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Identification of HER2-positive breast cancer patients with a high risk of developing brain metastases: a Portuguese single-center retrospective analysis

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LIST OF ABBREVIATIONS

AC-D - doxorubicin, cyclophosphamide and docetaxel

ALND- axillary lymph node dissection

ANEC - alive with no evidence of cancer

AWEC - alive with evidence of cancer

BC - breast cancer

BMFS - brain metastasis-free survival

BRCA1 - breast-cancer susceptibility 1 gene

BRCA2 - breast-cancer susceptibility 2 gene

CNS - central nervous system

DCIS - in situ ductal carcinoma

DNEC - dead with no evidence of cancer

DWEC - dead with no evidence of cancer

ECOG - Scale of Performance Status

L- lumpectomy

FEC-D - 5-fluorouracil, epirubicin, cyclophosphamide and docetaxel

M- mastectomy

MRM - modified radical mastectomy

OS - overall survival

RE - estrogen receptor

RP - progesterone receptor

SLNB- sentinel lymph node biopsy

Std - standard desviation

TNM - Classification of Malignant Tumors

SUMMARY

Introduction: Breast cancer (BC) represents one of the most frequent causes of brain metastases (BM). BM can develop in up to 10-16% of patients with metastatic breast cancer (MBC) during their disease course. Overexpression of human epidermal growth factor receptor 2 (HER2) is a well-known risk factor for BM development. Incidence of brain metastases can rise up to 40% is this BC subtype.

Objectives: Primary endpoint was to determine risk factors for BM in HER2-positive BC patients for the first time in a Portuguese population. Secondary endpoints were evaluation of brain metastasis-free survival (BMFS), overall survival (OS) and survival after brain metastization (SABM).

Material and Methods: Medical records of HER2-positive BC patients admitted at a single tertiary center, between 2007 and 2013, were retrospectively analyzed.

Descriptive analysis of main demographic, clinical-pathological characteristics and incidence of BM was performed. Chi-square, Fisher or T-Student tests were used to analyze the difference of proportions for statistical significance. Kaplan-Meier method was used to estimate BMFS and OS and patient groups were compared using log rank test. Simultaneous relationship of multiple prognostic factors was assessed using Cox's proportional hazard regression analysis. Differences were considered statistically significant when $p < 0.05$.

Results: Incidence of brain metastases in HER2-positive BC was 7%. HER2 positive BC patients with larger tumours (>2cm), node-positive disease (>3), TNM stage IV, median value of Ca15.3>25, and ECOG≥1 at diagnosis had a significantly higher incidence of brain metastases ($p<0.05$). Median BFMS was 25.8 months. Median OS in HER2-positive BC patients with BM was 40.3 months vs. 52.2 months in those without BM ($p<0.05$).

Conclusion: Brain metastases in HER2-positive breast cancer have high incidence and aggressiveness. Several clinicopathological parameters are able to predict brain metastization in HER2-positive BC patients.

KEY-WORDS

Breast cancer ▪ Brain metastasis ▪ HER2-positive ▪ Brain metastasis-free survival ▪ Overall survival

RESUMO

Introdução: A neoplasia maligna da mama (NMM) representa uma das causas mais frequentes de metástases cerebrais (MC). As metástases cerebrais podem desenvolver-se em 10-16 % dos doentes com NMM metastática durante o curso da doença. A sobre-expressão do recetor do fator de crescimento epidérmico humano 2 (HER2) é um fator de risco conhecido para o desenvolvimento de MC. A incidência de MC pode alcançar 40% neste subtipo de neoplasia da mama.

Objetivos: O objetivo primário foi determinar quais os fatores de risco para MC em doentes com NMM HER2-positiva, pela primeira vez na população portuguesa. Os objetivos secundários foram a avaliação da sobrevivência livre de metástases cerebrais (SLMC), a sobrevivência global (SG) e a sobrevivência após a metastização cerebral (SAMC).

Material e Métodos: Foram analisados retrospetivamente os prontuários de doentes com NMM HER2+ internados em um único centro terciário, entre 2007 e 2013. Realizou-se a análise descritiva das principais características demográficas e clínico-patológicas e determinou-se a incidência de metástases cerebrais na NMM HER2+. Utilizaram-se os testes Qui-quadrado, *Fisher* e *t-Student* para testar a diferença de proporções para significância estatística. O método de *Kaplan-Meier* foi usado para estimar SLMC e SG e os grupos de doentes foram comparadas pelo teste de *log rank*. A relação de múltiplos fatores prognósticos foi avaliada através de análise de regressão de riscos proporcionais de Cox. As diferenças foram consideradas estatisticamente significativas quando $p < 0,05$.

Resultados: A incidência de metástases cerebrais em doentes com neoplasia da mama HER2-positiva foi de 7%. Os doentes com tumores grandes (>2cm), gânglios linfáticos afetados (>3), estadio IV (TNM), valor da Ca15.3>25 e ECOG≥1 ao diagnóstico tiveram uma maior incidência de metástases cerebrais ($p < 0.05$). A mediana da SLMC foi de 25.8meses. A mediana da SG foi de 40,3meses versus 52.2 no grupo que não metastizou para o cérebro.

Conclusão: A metastização cerebral em doentes com neoplasia da mama HER2 positiva tem alta incidência e agressividade. Os fatores clínico-patológicos são importantes na previsão de metástases cerebrais nesses doentes.

PALAVRAS-CHAVE Neoplasia maligna da mama ▪ Metástases cerebrais ▪ HER2-positivo ▪ Sobrevivência livre de metástases cerebrais ▪ Sobrevivência global

INTRODUÇÃO: A NMM é a neoplasia mais frequentemente diagnosticada e a principal causa de morte por neoplasia entre as mulheres em todo o mundo[1][2], com uma estimativa de 1,7 milhões de casos e 521,900 mortes em 2012 [3]. Desde 2008, as estimativas têm revelado um aumento da incidência da neoplasia de mama em mais de 20% e da mortalidade em 14%.

Cerca de 6% dos doentes com NMM apresentam-se com metástases no momento do diagnóstico, implicando uma taxa de sobrevida aos 5 anos de 21%. Dependendo dos fatores prognósticos, até 30% dos doentes com gânglios negativos e até 70% de doentes com gânglios positivos ao diagnóstico irão recidivar[4]. Os dados revelam que, na recidiva, cerca de 10-30% serão loco-regionais, 60-70% metástases à distância e 10-30% ambos. Os locais mais comuns de metastização incluem o osso (mais frequente), seguido de pulmão, pleura, tecidos moles e fígado [5] [6] [7] [8] [9]. A metástase do sistema nervoso central (CNS) raramente é o primeiro local de recidiva[10]. Como o rastreio, por imagem, de metástases do CNS não é realizada rotineiramente em doentes assintomáticos, a incidência e relevância clínica de metástases ocultas no CNS na NMM avançada é desconhecida. Um trabalho prévio mostrou que em 155 estudos de imagem de triagem realizados, 15% evidenciavam metástases ocultas no SNC [11]. No entanto, este número pode subir até um terço dos doentes com NMM avançada[12]. Um estudo de autópsia revelou que as metástases do CNS estavam presentes em aproximadamente 30% dos doentes [13].

O oncogene HER2 está amplificado em NMM primárias e em linhas celulares humanas[14]. Os dados indicam que este gene pode desempenhar um papel no comportamento e/ou patogénese da NMM em humanos[14]. O HER2 é sobre-expresso em 25-30% de todas as NMM humanas [14] [15] com níveis de amplificação entre 2 a 20 vezes[14] .

Estudos retrospetivos indicaram que na NMM HER2+ pode haver um aumento de risco no desenvolvimento de metástases do CNS[16]. Na verdade, um grande estudo retrospetivo revelou que entre as doentes com NMM HER2+, a incidência cumulativa de doença do CNS em 10 anos foi maior do que entre as doentes com NMM HER2- (6,8% versus 3,5% em 10 anos; P<0,01)[16]. Além disso, no estudo epidemiológico registHER, das doentes com NMM portadoras de tumores confirmadamente HER2+ 37,3% tinham metástases no CNS, 7%, no momento do seu diagnóstico inicial de MC e 30% como um local subsequente da doença[17].

A presença de metástases viscerais e a não administração de trastuzumab no contexto metastático, parecem aumentar, aparentemente, a probabilidade de desenvolvimento de MC na NMM HER2+ avançada [18] [19]. Outros dados revelam que a MC como recidiva sintomática ocorre em aproximadamente em 39% de doentes com NMM HER2+ [18].

A idade média dos doentes que desenvolveram MC foi de 50 anos em uma análise retrospetiva alemã[20], 54 anos, num estudo japonês [21] e 52 anos num estudo asiático[22]. O tempo médio desde o tratamento até à recidiva cerebral em doentes com NMM HER2+ foi de 15 meses e a percentagem cumulativa a 1 ano, 3-anos e 5 anos é de 17, 42 e 55%, respetivamente[18]. O risco médio anual de MC para os doentes sobreviventes de NMM HER2+, durante 7 anos consecutivos de follow-up, foi de 10%[18]. Finalmente, a sobrevivência mediana após o desenvolvimento de MC em NMM HER2+ foi de 11,5 meses[21] [23].

A hipótese subjacente a este trabalho é que um conhecimento mais profundo dos fatores de risco para a MC em doentes com NMM HER2-positiva poderia permitir uma melhor seleção dos doentes para estratégias preventivas, que podem incluir terapêuticas-alvo e/ou terapêuticas mais eficazes com potencial para melhorar a sua eficácia.

Sendo assim, este estudo foi o primeiro a ser realizado numa população Portuguesa. O nosso objetivo foi determinar fatores de risco para MC em doentes portugueses com NMM HER2-positiva, avaliar a SLMC, SG e SAMC.

MATERIAIS E MÉTODOS: Foram analisados, retrospetivamente, os registos clínicos de doentes com NMM HER2-positiva admitidos no Instituto Português de Oncologia do Porto, entre 2007 e 2013. Os critérios de elegibilidade para a análise incluíram doentes com diagnóstico histológico de NMM HER2+, identificado por técnica imunohistoquímica ou hibridação *in situ* fluorescente (FISH), no tumor primário, recorrente ou metastático. Foram registadas as seguintes características clínico-patológicas: idade no momento do diagnóstico de NMM e de MC na NMM, a idade de metástase cerebral, status menopáusico, histologia do tumor e grau, presença de recetores de progesterona e/ou estrogénio, o status HER2, subtipo histológico de tumor, estadiamento TNM, tipo de cirurgia para a NMM, radioterapia, quimioterapia, terapia anti-HER2, terapia endócrina, localização da primeira recidiva/ progressão, local dominante da doença metastática (tecido visceral, tecido mole ou óssea), localização da primeira recidiva visceral extracraniana e cerebral (como primeira recidiva).

Realizou-se a análise descritiva das principais características demográficas e clínico-patológicas e determinou-se a incidência de MC na NMM Her2+. Utilizaram-se os testes Qui-quadrado, *Fisher* e *t-Student* para testar a significância estatística entre diferenças de proporções . A SLMC foi definida como o intervalo de tempo entre o diagnóstico de NMM e o diagnóstico de primeira MC. A SG foi definida como o intervalo de tempo entre o diagnóstico de NMM e a data de morte, por qualquer causa. A SAMC foi determinada como o intervalo de tempo entre o diagnóstico de MC e a data de morte, por qualquer causa. O método de *Kaplan-Meier* foi usado para estimar SLMC, SG e SAMC. Os grupos de doentes foram comparados pelo teste *log rank*. A relação de múltiplos fatores prognósticos foi avaliada através de análise de regressão de riscos proporcionais de Cox. As diferenças foram consideradas estatisticamente significativas quando p <0,05.

RESULTADOS: A população em estudo compreendeu 630 doentes com NMM HER2+. A Tabela I apresenta as principais características demográficas e clinico-patológicas desta população, a qual foi dividida em 2 grupos: o Grupo A que correspondeu a doentes com neoplasia da mama HER2+ sem metastização cerebral e o Grupo B que englobou doentes com neoplasia da mama HER2+ com metastização cerebral. Não houve diferenças na idade média entre os grupos [52,0 para o grupo A e 49,5 anos para o Grupo B, respetivamente (p> 0,05)]. Em ambos os grupos, a maioria das mulheres era pós-menopáusicas, multípara, sem história pessoal ou familiar de neoplasia de mama ou ovário. Os testes genéticos foram realizados em 103 doentes, verificando-se 2 casos com BRCA1 mutado e 6 casos com BRCA2 mutado no Grupo A (n = 99), sendo negativos todos os casos testados no Grupo B (n = 4) (P>0,05). Descobrimos um valor médio de Ca15.3 na altura do diagnóstico inferior no grupo A quando comparado com o Grupo B [19,0 vs. 28,0, respectivamente (p<0,05), Tabela I]. O tamanho do tumor foi significativamente diferente entre o Grupo A em comparação com o Grupo B [2,8 cm vs. 5,5 cm (p <0,05)]. Da mesma forma, o número médio de gânglios linfáticos afetados foi diferente [3 vs. 9, respetivamente, para o Grupo A e B (p <0,05)]. No Grupo A, a maioria dos doentes foram diagnosticadas em estadio IA, enquanto no Grupo B, a maioria dos casos foi estadiada como IIIA ou IV (p <0,05). A Figura 2 apresenta a distribuição dos casos no Grupo B e pela classificação TNM.

As Figuras 2 a 4 descrevem as características patológicas da população estudada, sem diferenças significativas entre os grupos. O tipo histológico mais frequente foi o carcinoma ductal invasor (84,6% no grupo A e 74,4 % no Grupo B, p> 0,05) e grau 3

(56,6% no grupo A e 56,4 % no Grupo B, p> 0,05). A proporção de casos com recetores hormonais foi semelhante em ambos os grupos [RE+ (74,7% no grupo A e 61,4 % no Grupo B, p> 0,05) e RP+ (60,1% no grupo A e 54,5% no Grupo B, p>0,05)]. A maioria dos tumores apresentou permeação linfática (51,0% no grupo A e 67,9% no Grupo B, p>0,05) e presença de carcinoma ductal *in situ* (89,6% no grupo A e 90 % no Grupo B, p>0,05), frequentemente de alto grau (82,9% no grupo A e 87,0 % no Grupo B , p>0,05). Encontramos uma diferença estatisticamente significativa no ECOG (Tabela I), sendo a proporção de doentes com ECOG 0 no Grupo A 8 vezes maior que no Grupo B (p <0,05). Os pacientes do Grupo B apresentaram doença mais avançada no momento do diagnóstico, em particular no estádio TNM IV (p< 0,05), como apresentado na Tabela I. As pacientes do Grupo B apresentaram uma probabilidade 12 vezes superior de apresentar metastização ao diagnóstico, comparativamente com o Grupo A (OR = 11,5 95 % CI 4,2-31,3).

Os locais mais comuns de metástases ao diagnóstico foram osso, fígado, gânglios linfáticos, pulmão, pleural e cérebro, em ordem de frequência, conforme apresentado na Tabela II. Os doentes NMM HER2+ com metastização óssea (OR = 13,7, IC 95% 4,7-39,7), ganglionar (OR = 9,3, IC 95 % 1,5-56,9) ou hepática (OR = 9,7, IC 95% 2,6-35,7) no momento do diagnóstico tiveram um maior risco de desenvolver MC.

Após o diagnóstico de NMM, a cirurgia foi realizada como o primeiro tratamento em 83% dos doentes no grupo A (p<0,05) e a quimioterapia em 61 % doentes no Grupo B (p<0,05). O tipo de cirurgia mais realizada foi mastectomia com ou sem esvaziamento axilar, em ambos os grupos, embora tenha existido uma tendência para mastectomia com esvaziamento axilar (MRM) no Grupo B (64,7% no grupo A e 97,5 % no Grupo B, quando a cirurgia foi indicada). O tipo de quimioterapia foi diferente entre os grupos. No Grupo A maioria dos doentes (82%) foi submetida ao esquema FEC-D com intenção adjuvante, enquanto no Grupo B a maior parte fez o esquema AC-D, neoadjuvante (54 %) (p<0,05). Em ambos os grupos, o tratamento foi reforçado com radioterapia adjuvante, terapia hormonal adjuvante e trastuzumab, como mostrado na Tabela III. Os doentes com NMM HER2+ que desenvolveram MC eram mais propensos a receber esses tratamentos com intenção paliativa após o diagnóstico (p <0,05).

A Figura 5 mostra o tipo de recidiva ou progressão após primeiro tratamento. O risco de recidiva foi 76 vezes maior (OR = 76,2 IC 95 % 31,9-182,3) e o de progressão 37 vezes maior (OR= 36,7 IC 95% 9,1-148,0) em doentes com NMM HER2+ que desenvolveram metástases em outros locais além do SNC. Os doentes HER2+ sem MC e com MC

tiveram em comum os locais mais comuns de primeira recidiva ou progressão com algumas diferenças na frequência. As metástases ósseas foram o local mais comum em doentes HER2* sem MC, 58,5 %, enquanto as MC foram o local mais comum em doentes HER2+ com MC,56,8%. A Tabela IV apresenta a frequência relativa da localização de recidiva ou progressão nos Grupos A e B.

Quarenta e quatro doentes foram diagnosticados com metástases no SNC. A maioria dos casos apresentou sintomas neurológicos (94%) e teve uma ECOG≥1 (93%). O SNC como o primeiro local de metastização ocorreu em 61% dos doentes (n = 27). A maioria (69%) tinha 3 ou mais metástases. O local mais comum de metástases incluiu ambos os hemisférios (81,6%), cerebelo (57,9%), tronco cerebral (10,5%) e meninges (2,6%) (Figura 6). Ao diagnóstico de MC, metade dos doentes eram previamente metastáticos (N=22). Os locais mais comuns foram osso (29,5%), fígado (20,5%), sistema linfático (18,2%), pulmão (13,6%), pleura (11,4%), peritoneu (2,3%) e tecidos moles (2,3%) (Figura 7). Ao diagnóstico de MC, 16% dos doentes foram sincronicamente diagnosticados com outros locais de metastização: pulmonar (11,4%), ganglionar (6,8%), pleural (6,8%), óssea (4,5%), hepática (2,3%) e meníngea (2,3%) (Figura 8). No diagnóstico de metástases do SNC, 27% dos doentes estavam sob tratamento quimioterápico (Figura 9); 48% sob terapia hormonal (Figura 10) e 61% sob anti-HER2 (Figura 11).

Após o diagnóstico de MC, a maioria dos doentes (57%) foi submetida unicamente a radioterapia total do cérebro (WBRT). A neurocirurgia ou radiocirurgia seguida por WBRT foi realizada em 18% dos doentes (Figura 12). Menos de metade dos doentes foi submetida a tratamento adicional com quimioterapia (49 %) ou a terapia hormonal (44 %) após tratamento local (Figuras 13 e 14). O tratamento sistémico sem tratamento local prévio foi realizado em 7% dos doentes. Trastuzumab foi administrado a 40% dos doentes, lapatinib a 34,9 % e pertuzumab a 2,3% (Figura 15). Tratamento sintomático foi oferecido a 13,6 % dos doentes.

No último mês antes da morte, 1 doente realizou neurocirurgia, 2 doentes estavam em radioterapia, e a maioria dos doentes (75%) estavam sem qualquer tratamento sistémico e sob cuidados sintomáticos, apenas.

A sobrevivência mediana livre de metástases cerebrais em doentes com NMM HER2+ foi de 25,5 meses 95% IC 21,8-29,2. A Figura 16 mostra a curva de sobrevivência e a Tabela V descreve as variáveis clínico-patológicas e o seu possível significado prognóstico nesse grupo de doentes. As figuras 17 a 25 descrevem as curvas de BMFS

nas variáveis clínico-patológicas acima enumeradas. BMFS foi menor nos doentes com valor de Ca15.3>25; com tamanho do tumor>2cm; com número de gânglios linfáticos afetados>3; estadio TNM IV; com metastização óssea, ganglionar ou hepática no momento do diagnóstico de NMM, mas sem diferença estatisticamente significativa. Em doentes com NMM HER2+ com MC, SG foi de 40,3 ± 21,1 meses e de 52,2 ± 8,4 meses (p <0,05 IC 95% 0,8-12,2) no grupo sem MC (Figura 16). A sobrevivência mediana após metastização cerebral foi de 18 meses.

DISCUSSÃO: Neste estudo, a incidência de MC na NMM HER2+ foi de 7%, o que está de acordo com um estudo retrospetivo de 9.524 doentes com neoplasia da mama HER2+[16]. Da mesma forma, a idade mediana de 51 anos ao diagnóstico de MC foi a mesma encontrada em estudos alemão, japonês e asiático[20], [21], [22]. A nossa pesquisa revelou que 94% dos doentes com NMM HER2+ têm sintomas quando do diagnóstico de MC, o que contrasta com 39% mencionado num outro estudo[18]. Esta discrepância poderá ser explicada pelo uso mais frequente (e provavelmente excessivo) de exames de imagem cerebral em doentes assintomáticos, nessas séries. As MC como primeira manifestação de recidiva da doença ocorreram em 61% dos casos NMM HER2+ e a maioria deles teve mais de 3 metástases (69%), à semelhança de outros estudos que revelam múltiplas MC como a regra[20] [22] [24]. Os locais mais comuns foram os hemisférios cerebrais (53%), cerebelo (37%), tronco cerebral (7%) e meninges (2%). A mesma ordem de frequência foi encontrada num outro estudo, mas com maior incidência nos hemisférios (79%), menor no cerebelo (9%) e tronco cerebral (2%) [20]. No estudo registHER, 37,3% das doentes com tumores HER2+ tiveram MC, 7%, no momento do seu diagnóstico metastático e 30% como um local subsequente de doença[17]. No nosso estudo, 50% das doentes com NMM HER2+ foram previamente metastizadas noutros locais, nomeadamente, osso, fígado, gânglios linfáticos, pulmão, pleura, peritoneu e tecidos moles. Aquelas doentes que estavam metastizadas simultaneamente em outros locais, no momento do diagnóstico de MC, diferiram das anteriores por ordem de frequência: pulmão, gânglios linfáticos, pleura, osso, fígado e meninge. Dados de outros estudos mostraram locais de doença metastática semelhantes[20] [24]. No entanto, estes estudos não relataram se as metástases eram anteriores ou síncronas relativamente às MC.

Tentamos identificar os fatores de risco para o desenvolvimento de MC em doentes com NMM HER2+. A idade mais jovem (<35 anos), geralmente considerada um fator de risco

para recidiva cerebral na população de NMM em geral, não foi demonstrada[25]. No nosso estudo, a idade mediana dos doentes foi de 51 anos. Da mesma forma o estado hormonal, gravidez prévia, a história pessoal/familiar de neoplasia de mama ou ovário ou o IMC não foram preditivos de recidiva cerebral. No entanto, este estudo demonstrou, pela primeira vez, uma diferença significativa entre os dois grupos no que diz respeito ao *Performance status*, pois doentes com ECOG≥1 após diagnóstico de NMM HER2+ apresentaram maior risco de desenvolver MC. Os nossos dados revelaram, também, que os doentes com tumores de maiores dimensões e gânglios linfáticos metastizados tinham significativamente maior incidência de MC, o que foi concordante com outras séries publicadas [25] [26]. Contudo, o grau tumoral 3 não teve nenhuma influência, ao contrário do verificado em outros estudos [16]. Tumores HER2+/ER- estão associados a um maior desenvolvimento de MC [26] [24]. No nosso estudo, não encontramos nenhuma diferença em relação ao *status* do recetor hormonal. Igualmente, o tipo histológico, a permeação linfática ou presença de DCIS não foram indicadores de uma maior incidência de MC. Curiosamente, o marcador tumoral Ca15.3 encontrava-se significativamente mais elevado no momento do diagnóstico em doentes com NMM HER2+ que desenvolveram MC. Este dado está em acordo com uma maior incidência de MC encontradas em doentes com TNM IV nesta subpopulação, no momento de diagnóstico de NMM. Metástases ósseas, ganglionares e hepáticas ao diagnóstico em doentes com NMM HER2+ também foram associados maior incidência de MC. Esta descoberta levanta a hipótese de o CNS representar um “local santuário” para aqueles doentes que tiveram a doença controlada com terapia sistémica inicial, a qual habitualmente não penetra no SNC[27].

Após o diagnóstico de MC, a maioria dos doentes foram submetidos a WBRT apenas (57%) ou a neurocirurgia/radiocirurgia seguida por WBRT (18%). A maioria das doentes recebeu terapia anti-HER2 (77%). Aproximadamente metade das doentes foi submetida a quimioterapia (49%) ou a terapia hormonal (44%) como primeiro tratamento sistémico após MC. Num estudo clínico, radioterapia (93%) e quimioterapia (57%) foram os tratamentos mais comuns após o diagnóstico de MC. No entanto, a terapia anti-HER2 e a neurocirurgia (13%) foram oferecidas na mesma proporção que no nosso estudo[22].

A maioria dos doentes com NMM HER2+ com MC não estava a realizar qualquer terapêutica antineoplásica (local ou sistêmica) no último mês de vida. Na verdade, 75% dos doentes já se encontrava em cuidados sintomáticos, em linha com as boas práticas clínicas. Num estudo prévio, a causa de morte foi a progressão da MC em 35% dos

doentes, a progressão da doença sistémica em 30% e não foi especificada em 35% dos doentes[20]. Outros estudos revelaram que 55% dos doentes morreram devido a complicações da MC[22]. No nosso estudo, 86% dos doentes com NMM HER+ e MC morreram com evidência de cancro, mas não foi possível determinar se foi devido à metastização cerebral ou à progressão da doença sistémica.

Nesta série, a SLMC foi de 25,5 meses, semelhante ao encontrado num outro estudo (30,9 meses)[28]. A SG em doentes com NMM HER2+ e MC foi de 40,3 meses, significativamente inferior à dos doentes que não desenvolveram MC (52 meses), estando em linha com outros estudos [20] [18]. Adicionalmente, a SAMC mediana em doentes com NMM HER2+ (18 meses) foi superior à de outros estudos, que referem 10 meses [21]. A sobrevivência mais reduzida nestes estudos mais antigos é provavelmente explicada pela não utilização de trastuzumab. No entanto, uma SAMC de 18 meses foi semelhante à verificada em doentes que realizaram tratamento com trastuzumab, como no nosso estudo.

CONCLUSÃO: A incidência de MC em doentes NMM HER2+ nesta população portuguesa foi semelhante a outros estudos. O nosso estudo demonstrou que os fatores clínico-patológicos foram importantes na previsão de MC em doentes HER2+. Pela primeira vez foi identificado que pior *Performance status* e valores mais elevados de Ca15.3 no momento do diagnóstico de NMM estão associados a um maior risco de desenvolver MC. No entanto, serão necessários estudos adicionais, nomeadamente prospetivos, para validar estes novos resultados. A SAMC foi maior que noutros estudos, provavelmente devido ao tratamento com trastuzumab. No entanto, a SG em doentes com NMM HER2+ com MC foi menor do que em doentes que não tinham MC e investigação adicional é requerida para desenvolver terapêuticas mais eficazes.

INTRODUCTION

Breast cancer (BC) is the most frequently diagnosed malignant neoplasm and the leading cause of cancer death among females, worldwide [1][2], with an estimated 1.7 million cases and 521,900 deaths in 2012[3]. Based on 2008 estimates, BC incidence has increased by more than 20% and mortality has increased by 14%[2].

Approximately 6% of BC patients are metastatic at diagnosis with a 5-year survival rate of 21%. Depending on prognostic factors, up to 30% of node-negative and up to 70% of node-positive BC will relapse [4]. Published data revealed that upon relapse, about 10-30% will have loco-regional relapse, 60-70% distant metastasis, and 10-30% both. Most common sites of first metastasis are bone, then lung, pleura, soft tissue and liver[5][6][7] [8][9].

Central nervous system (CNS) metastasis is rarely the first manifestation of recurrence[10]. As screening CNS imaging is not routinely performed in asymptomatic patients, the incidence and clinical relevance of occult CNS metastases in advanced BC is unknown. A report showed that among 155 screening imaging studies performed, 15% disclosed occult CNS metastases[11]. However this number can rise up to one third of patients with advanced BC[12]. An autopsy study further revealed that CNS metastases were noted in approximately 30% of patients[13].

HER2 oncogene is amplified in subsets of primary BC and human BC cell lines[14]. Data indicates that this gene may play a role in biologic behavior and/or pathogenesis of human BC[14]. HER2 is overexpressed in 25-30% of all human BC [14] [15] with amplification levels from 2- to more than 20-fold[14].

Retrospective studies have indicated that HER2-positive status may increase the risk for developing CNS metastasis[16]. Indeed, a large retrospective study with early BC revealed that among HER2-positive patients, 10-year cumulative incidence of CNS disease was higher than in patients with HER2-negative BC (6.8% versus 3.5% at 10 years; $P < 0.01$) [16]. Moreover, in the epidemiological registHER study, BC patients who had confirmed HER2-positive tumors, 37.3% harbored CNS metastases, 7% at the time of their initial MBC diagnosis and 30% as a subsequent site of disease[17].

The presence of visceral metastases and lack of trastuzumab administration in the metastatic setting apparently increases the likelihood of early BM in advanced HER2-positive breast cancer[18][19]. Other data reveals that symptomatic brain relapse occurs approximately in 39% of HER2-positive BC patients[18].

Median age of patients who developed BM was 50 years in a German retrospective analysis [20], 54 years in a Japanese study [21] and 52 years in an Asian research[22]. Median time from treatment completion to brain relapse in HER2-positive BC patients s 15 months and cumulative 1-year, 3-year and 5-year risk of brain relapse is 17, 42 and 55%, respectively[18]. Average annual risk of brain relapse for surviving HER2-positive BC patients during consecutive 7 years of follow-up is 10%[18]. Finally, median survival after developing BM in HER2-positive BC is 11.5 months[21] [23].

We hypothesized that a more profound knowledge of risk factors for BM in HER2-positive BC patients might allow for better selection of patients for preventive strategies, which may include targeted or/and more effective therapeutics with potential to improve outcome.

Importantly, this study is the first to characterize a Portuguese population. Thus, our aim was to determine risk factors for BM in HER2-positive Portuguese BC patients, with evaluation of brain metastasis-free survival (BMFS), overall survival (OS) and survival after brain metastization (SABM).

MATERIAL AND METHODS

Study Population

Medical records of consecutive HER2-positive breast cancer patients admitted at *Instituto Português de Oncologia do Porto* between 2007 and 2013 were retrospectively reviewed.

Eligibility criteria included patients with histologically confirmed diagnosis of invasive HER2-positive BC, identified by immunohistochemistry or fluorescent *in situ* hybridization (FISH) analysis, either in primary, recurrent or metastatic tumor.

The following clinical-pathological characteristics were recorded: age at diagnosis, age at brain metastasis, menopausal status, tumor histology and grade, estrogen and progesterone receptor status, HER2 status, tumor subtype, TNM staging, breast cancer surgery, radiotherapy, chemotherapy, anti-HER2 therapy, endocrine therapy, location of first relapse/progression, dominant site of metastatic disease (visceral, soft tissue, bones), location of first extra-cranial visceral relapse and brain as first relapse.

Statistical analyses

Descriptive analysis of main demographic, clinical-pathological characteristics and incidence of BM were performed. Chi-square, Fisher and T-Student tests were used to test the difference of proportions for statistical significance. BMFS was measured as the interval from diagnosis of BC diagnosis until diagnosis of brain metastases. OS was defined as the interval from the date of primary BC diagnosis until death of any cause. SABM was determined as the interval from the date of BM diagnosis until death of any cause. Kaplan-Meier method was used to estimate BMFS, OS and SABM. Patient groups were compared using log rank test. Multiple prognostic factors were assessed using Cox's proportional hazard regression analysis. Differences were considered statistically significant when $p < 0.05$.

RESULTS

I – MAIN DEMOGRAPHIC AND CLINICAL-PATHOLOGICAL DATA

Overall, 630 patients were enrolled in this study. Table I and Figure 1 describe main demographic, clinical-pathological of the studied population, which was divided into Group A – HER2-positive BC patients without CNS metastases, and Group B - HER2-positive BC patients with CNS metastases.

Table I- Main demographic, clinical-pathological of the studied population. Group A – HER2-positive BC patients without CNS metastases; Group B – HER2-positive BC patients with CNS metastases. Statistical test for comparison of continue variables – T-test and categorical variables – Chi square and Fisher-test; ANEC – alive with no evidence of cancer; AWEC – alive with evidence of cancer; DNEC – dead with no evidence of cancer; DWEC – dead with evidence of cancer.

Clinical Variable	Group A	Group B	OR	95% CI of ratio	P
Age (years)	N=586	N=44			
	52	49.5	0.69	0.2-2.0	0.524
Body Mass Index n (%)	N=501	N=35			
Normal <25Kg/m^2	209(41.7)	15(42.9)	0.95	0.5-1.9	0.895
High>25kg/m^2	292(58.3)	20(57.1)			
Hormonal Status n (%)	N=562	N=37	0.71	0.4-1.4	
Pre-menopausal	226 (40.2	18(48.6)			0.312
Post-menopausal	336 (59.8)	19(51.4)			
Prior Pregnancy n (%)	N=528	N=37	0.71	0.3-1.8	
Nulliparous	64(12.1)	6(16.2)			0.441
Multiparous	464(87.9)	31(83.8)			
Personal History n (%)	N=521	N=30	0.74	0.1-5.7	1.000
	23(4.4)	1(3.3)			
Family History n (%)	N=470	N=30	1.10	0.5-2.4	0.806
	162 (34.5)	11(36.7)			
RE+ n (%)	N=586	N=44	0.54	0.3-1.0	0.052
	438 (74.7)	27(61.4)			
RP+ n (%)	N=586	N=44	0.80	0.4-1.4	0.471
	352(60.1)	24(54.5)			
Grade n (%)	N=562	N=39			
1	11(2.0)	0(0)			
2	233(41.5)	17(43.6)	-	-	-
3	318(56.6)	22(56.4)			

Lymphovascular invasion n (%)	N=396 202(51.0)	N=28 19(67.9)	2.03	0.9-4.6	0.116
Presence of DCIS	N=450 407(89.6)	N=30 27(90.0)	1.04	0.3-3.4	0.951
Grade of DCIS n (%) Low Intermediate/High	N=391 26(6.6) 365(93.4)	N=23 1(4.3) 22(95.7)	1.57	0.2-12.1	1.000
BRCA Status n (%) Negative BRCA 1/2	N=8 2(25.0) 6(75.0)	N=0 0(0) 0(0)	-	-	-
ECOG at diagnosis n (%) 0 ≥1	N=381 334(87.7) 47(12.3)	N=17 8(47.1) 9(52.9)	7.99	2.9-21.7	0.000
Median value of Ca15.3 n (%) <30 >30	N=586 435(74.2) 151(25.8)	N=44 21(47.7) 23(52.3)	3.16	1.7-5.9	0.000
Tumor Size n (%) <2cm >2cm	N=586 264(45.1) 322(54.9)	N=44 2(4.5) 42(95.5)	17.22	4.1-71.8	0.000
No. of lymph nodes metastasized n (%) <3 >3	N=586 411(70.3) 174(29.7)	N=44 10(22.7) 34(77.3)	8.03	3.9-16.6	0.000
TNM stage classification n (%) I,II,III IV	N=519 505(97.3) 14(2.7)	N=29 22(75.9) 7(24.1)	11.48	4.2-31.3	0.000
Status at last observation n (%) ALIVE DEAD	N=586 559(95.4) 27(4.6)	N=44 10(22.7) 34(77.3)	70.39	31.5-157.3	0.000
ANEC AWEC DNEC DWEC	533(91.0) 26(4.4) 6(1.0) 21(3.6)	0(0) 10(22.7) 0(0) 34(77.3)	-	-	-

There were no differences in median age between groups, with 52.0 years for Group A and 49.5 years for Group B, respectively ($p>0.05$). Most women were post-menopausal, multiparous, and had no personal or family history of breast or ovarian cancer, in both groups. Genetic testing was performed in 103 patients, disclosing 2 cases with BRCA1

mutations and 6 cases with BRCA2 mutations in Group A (N=99), whereas all cases were negative among the small number of patients tested in Group B (N=4) (p>0.05).

A lower median value of Ca15.3 at diagnosis in Group A compared to Group B was uncovered [19.0 vs 28.0, respectively (p<0.05)].

Tumor size significantly differed between Group A and Group B [2.8 cm vs. 5.5 cm (p<0.05)]. Similarly, the number of metastasized lymph nodes was higher in group B [3 vs. 9, respectively for Groups A and B, (p<0.05)]. In Group A, most of patients were stage IA, while in Group B most cases were at stages IIIA or IV (p<0.05). Figure 2 depicts the distribution of cases in Groups A and B according to TNM classification.

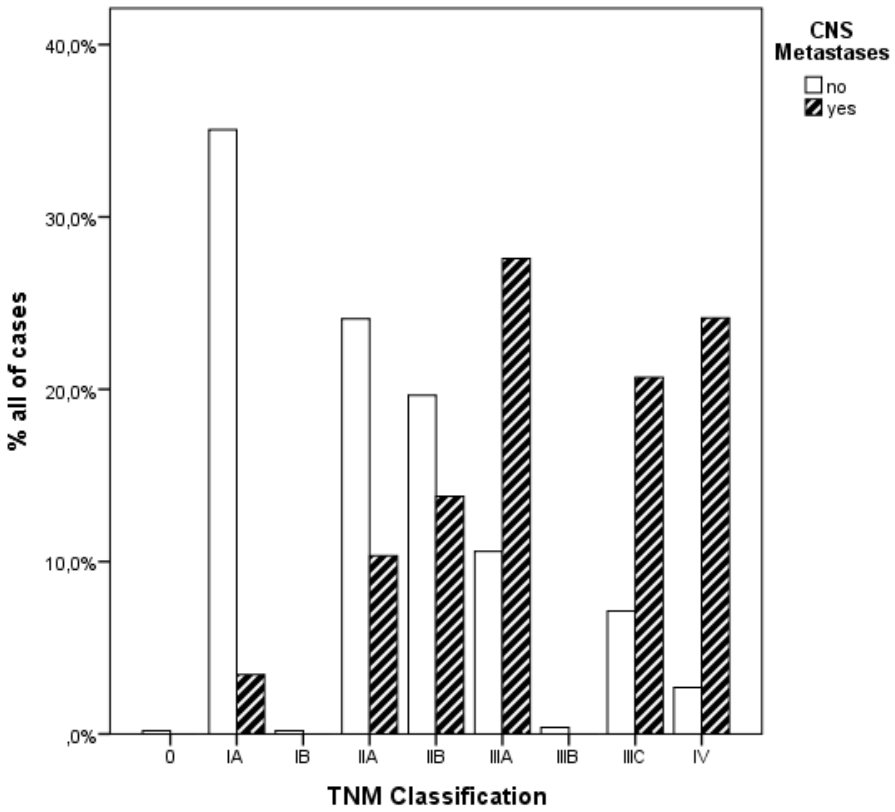

Figure 1- Distribution of cases of Group A and B by TNM classification. In Group A most cases stage IA (35.1%); in Group B the most of cases were stage IIIA (27.6%) and stage IV (24.1%).

Figures 2 to 4 describe the pathological features of the studied population, with no significant differences between groups. Most frequent histological type was invasive ductal carcinoma (84.6% in Group A and 74.4% in Group B, p>0.05) and grade 3 (56.6% in Group A and 56.4% in Group B, p>0.05). The frequency of hormonal receptors positivity in both groups was similar, as well [RE+ (74.7% in Group A and 61.4% in Group B, p>0.05) and RP+ (60.1% in Group A and 54.5% in Group B, p>0.05)]. Most cases presented lymphovascular invasion (51.0% in Group A and 67.9% in Group B, p>0.05) and presence of DCIS (89.6% in Group A and 90% in Group B, p>0.05), mostly high-grade (82.9% in Group A and 87.0% in Group B, p>0.05).

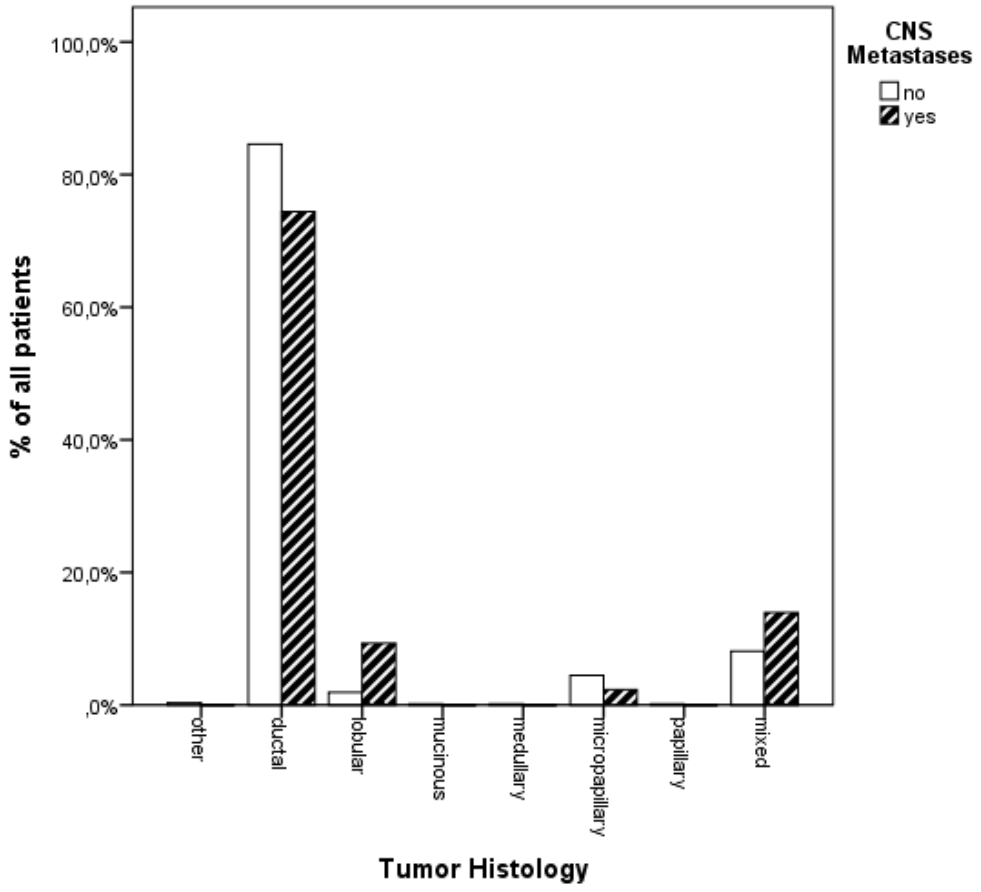

Figure 2- Distribution of cases of Group A and B by tumor histology (N=621, p>0.05).

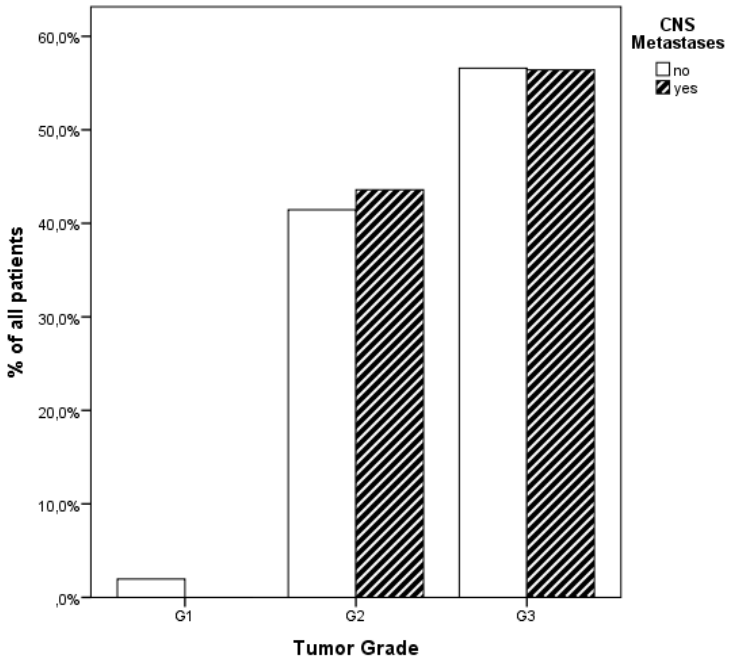

Figure 3- Distribution of cases of Group A and B by tumor grade (N=601, p>0.05).

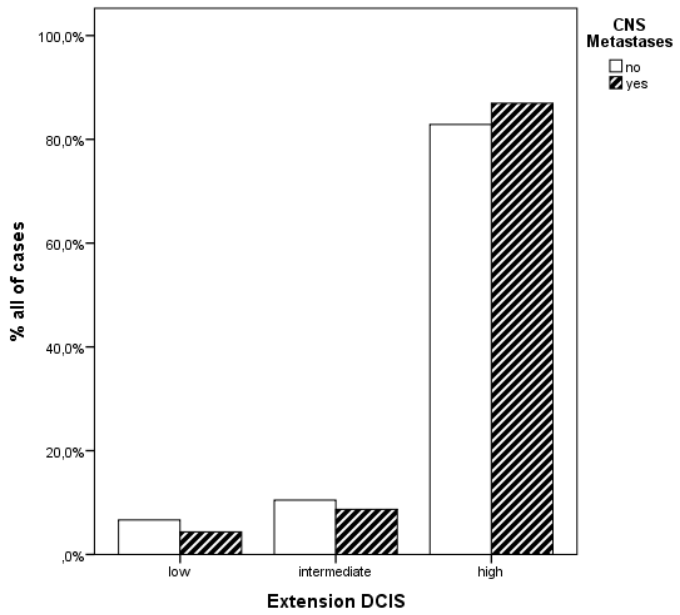

Figure 4- Distribution of cases of Group A and B by extension of DCIS (N=414, p>0.05).

We found a statistical significant difference in ECOG: Group A patients had a better performance status (ECOG=0) compared with Group B (ECOG≥1) (p<0,05).

Patients in Group B presented more advanced disease at diagnosis, with a higher proportion of TNM stage IV disease (p<0.05). Group B patients had a likelihood of being metastasized at diagnosis approximately 12 times higher than those of Group A (OR= 11.5 95% CI 4.2-31.3).

Most common sites of metastases at diagnosis were bone, liver, lymph node, pulmonary, pleural and brain, in order of frequency, as presented in Table II. HER2-positive BC patients with bone (OR=13.7 95% CI 4.7-39.7), lymph node (OR=9.3 95% CI 1.5-56.9) and liver metastasis (OR=9.7 95% CI 2.6-35.7) at diagnosis had a higher risk of developing BM.

Table II- Distribution of metastases location in Group A and B at diagnosis of breast cancer.

	Group A N=586	Group B N=44	OR	95% CI of ratio	p
Bone Metastasis n (%)	8(1.4)	7(15.9)	13.67	4.7-39.7	0.000
Lymph node Metastasis n (%)	3(0.5)	2(4.5)	9.25	1.5-56.9	0.042
Pulmonary Metastasis n (%)	3(0.5)	0(0)	0.93	0.9-1.0	1.000
Liver Metastasis n (%)	6(1.0)	4(9.1)	9.67	2.6-35.7	0.003
Pleural Metastasis n (%)	0(0)	1(2.3)	0.07	0.05-0.09	0.070
Brain Metastasis n (%)	0(0)	1(2.3)	0.07	0.05-0.09	0.070
Lymphangitis Carcinomatosis, Meingeal,Peritoneal, Soft Tissues or Other Metastases n (%)	0(0)	44(0)	-	-	-

Upon BC diagnosis, surgery as the first treatment for 83% of patients in Group A (p<0,05), and chemotherapy for 61% in Group B (p<0,05). Type of surgery most often performed was mastectomy ± axillary lymph node dissection (ALND), in both groups, although there was a trend in Group B for mastectomy with lymph node dissection (MRM) (64.7% in Group A and 97.5% in Group B, when surgery was indicated). Type of chemotherapy was different between groups. In Group A, most patients (82%) underwent FEC-D with adjuvant intention while in Group B most of them were submitted to neodjuvant AC-D (54%) (p<0.05).

In both groups, treatment was reinforced with adjuvant radiotherapy, adjuvant hormonal therapy if positive hormonal receptors, and trastuzumab, as shown in Table III. HER2-positive BC patients that underwent treatments with palliative intention after diagnosis had a greater chance of developing BM (p<0.05).

Table III- Distribution of cases the Group A and Group B by types of treatment.

	Group A	Group B	OR	95% CI of ratio	p
First Treatment n (%)	N=585	N=44	8.0	4.2-15.2	0.000
Surgery	488(83.4)	17(38.6)			
Chemotherapy	97(16.6)	27(61.4)			
Kind of surgery n (%)	N=575	N=40	21.82	2.9-156.0	0.000
L+SLNB and L+ALND	203(35.3)	1(2.5)			
M+SLNB and MRM	372(64.7)	39(97.5)			
Radiotherapy n (%)	N=455	N=35	7.21	2.1-25.3	0.007
Neoadjuvant/Adjuvant	447(98.2)	31(88.6)			
Palliative	8(1.8)	4(11.4)			
Chemotherapy Intention n (%)	N=580	N=43	10.06	3.7-27.5	0.000
Neoadjuvant/Adjuvant	569(98.1)	36(83.7)			
Palliative	11(1.9)	7(16.3)			
Kind of Chemotherapy n (%)	N=533	N=38	6.51	3.3-12.9	0.000
FEC-D	440(82.6)	16(42.1)			
AC-D	93(17.4)	22(57.9)			
Trastuzumab n (%)	N=579	N=43	10.80	4.1-28.1	0.000
Neoadjuvant/Adjuvant	567(97.9)	35(81.4)			
Palliative	12(2.1)	8(18.6)			
Hormonal Therapy intention n	N=443	N=27			

(%)	2(0.5)	0(0)	-	-	-
Neoadjuvant	432(97.5)	23(85.2)			
Adjuvant	9(2.9)	4(14.8)			
Palliative					
Kind of hormonal therapy n (%)	N=584	N=43			
No	142(24.3)	17(39.5)			
Tamoxifen	184(31.5)	17(39.5)			
Anastrozole	145(24.8)	7(16.3)			
Letrozole	9(1.5)	2(4.7)	-	-	-
Switch	77(13.2)	0(0)			
Extended	26(4.5)	0(0)			
Other	1(0.2)	0(0)			

Figure 5 shows the type of relapse or progression after first treatment. Risk for relapse was 76 times higher (OR=76.2 95% CI 31.9-182.3) and risk of progression was 37 times higher (OR=36.7 95% CI 9.1-148.0) in HER2-positive BC patients who developed BM in other sites besides CNS.

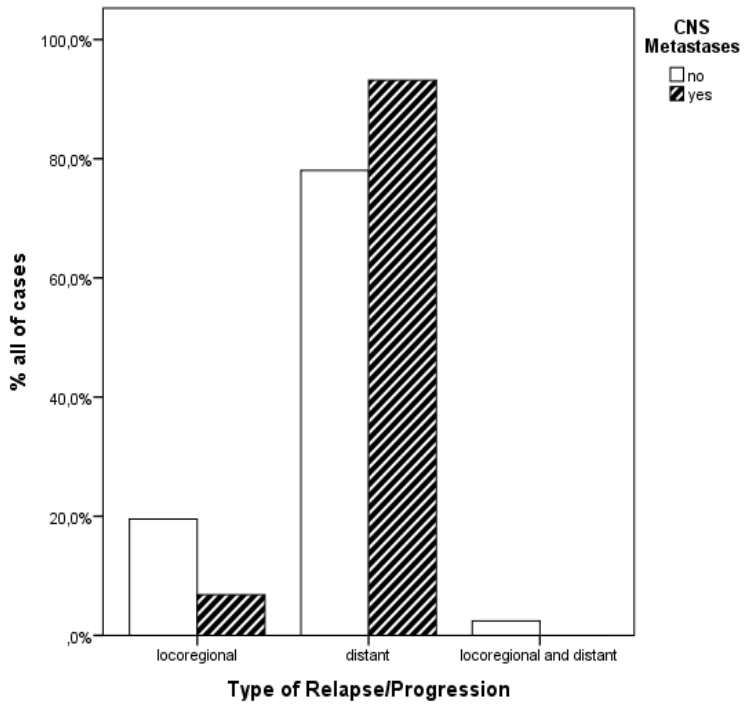

Figure 5- Distribution of cases in both type of first relapse or progression. Most cases in both groups had a distant relapse or progression.

HER2-positive patients with no BM and with BM had similar sites of first relapse or progression, with some differences in frequency. Bone metastases were the most common site in HER2-positive patients with no BM (58.5%), while BM were the most common site in HER2-positive patients with CNS involvement (56.8%). Table IV presents the relative frequencies of relapse or progression localization in Groups A and B.

Table IV- Distribution of cases the Group A and Group B by the location of first relapse or progression.

	Group A N=41	Group B N=44	OR	95% CI of ratio	p
Bone Metastasis n (%)	24(58.5)	8(18.2)	0.16	0.1-0-4	0.000
Lymph node Metastasis n (%)	9(22.0)	6(13.6)	0.56	0.2-1.7	0.398
Pulmonary Metastasis n (%)	8(19.5)	8(18.2)	0.92	0.3-2.7	1.000
Liver Metastasis n (%)	8(19.5)	6(13.6)	0.65	0.2-1.4	0.564
Pleural Metastasis n (%)	5(12.2)	5(11.4)	0.92	0.2-3.5	1.000
Brain Metastasis n (%)	0(0)	25(56.8)	0.32	0.2-0.5	0.000
Meningeal Metastasis n (%)	0(0)	1(2.3)	0.51	0.4-0.6	1.000
Peritoneal Metastasis n (%)	1(2.4)	1(2.3)	0.93	0.1-15.4	1.000
Soft Tissue Metastasis n (%)	0(0)	1(2.3)	0.51	0.4-0.6	1.000
Other Metastasis n (%)	7(17.1)	4(9.1)	0.49	0.1-1.8	0.341
Lymphangitis Carcinomatosis n (%)	0(0)	0(0)	-	-	-

II – CHARACTERIZATION OF HER2+ CNS METASTIC POPULATION

Forty-four patients were diagnosed CNS metastases. Most of cases presented neurological symptoms (94%) and had an ECOG≥1 (93%). Brain metastases as first site of metastasis occurred in 61% of patients (N=27). Most (69%) had 3 or more metastases and the most common sites of metastasis were both hemispheres (81.6%), cerebellum (57.9%), brainstem (10.5%) and meningeal (2.6%) (Figure 6).

At diagnosis of BM, half of the patients were previously metastatic (N=22). Most common sites were bone (29.5%), liver (20.5%), lymph node (18.2%), pulmonary (13.6%), pleural (11.4%), peritoneal (2.3%) and soft tissues (2.3%) (Figure 7).

At diagnosis of BM, 16% of patients were synchronously diagnosed other sites of metastases: pulmonary (11.4%), lymph node (6.8%), pleural (6.8%), bone (4.5%), liver (2.3%) and meningeal metastases (2.3%) (Figure 8).

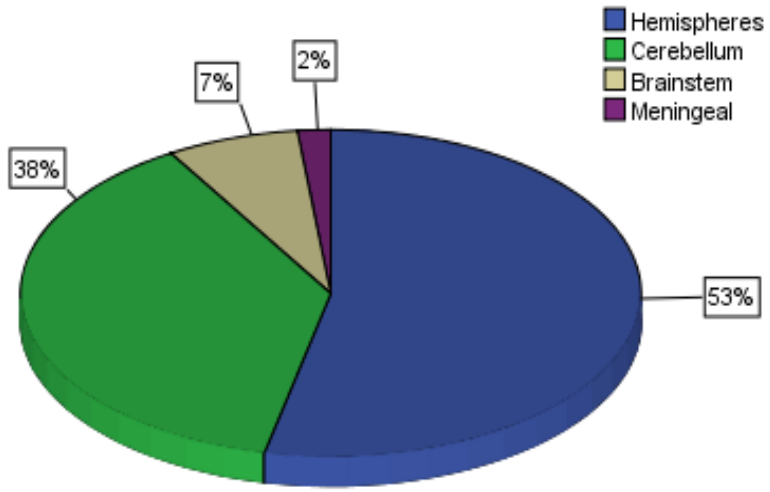

Figure 6- Distribution of cases by site of brain metastases (%, relatively all metastases N=58).

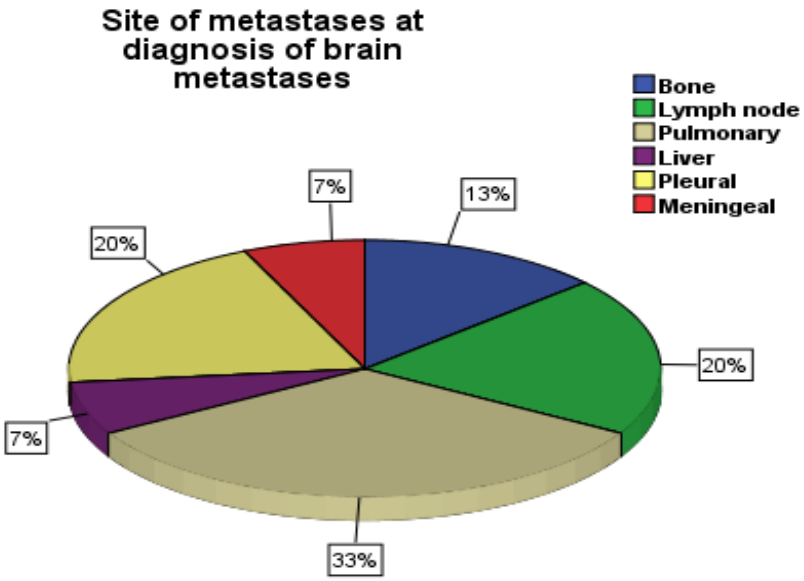

Figure 7- Distribution of cases by site of prior metastases before brain metastases (%, relatively all metastases N=45).

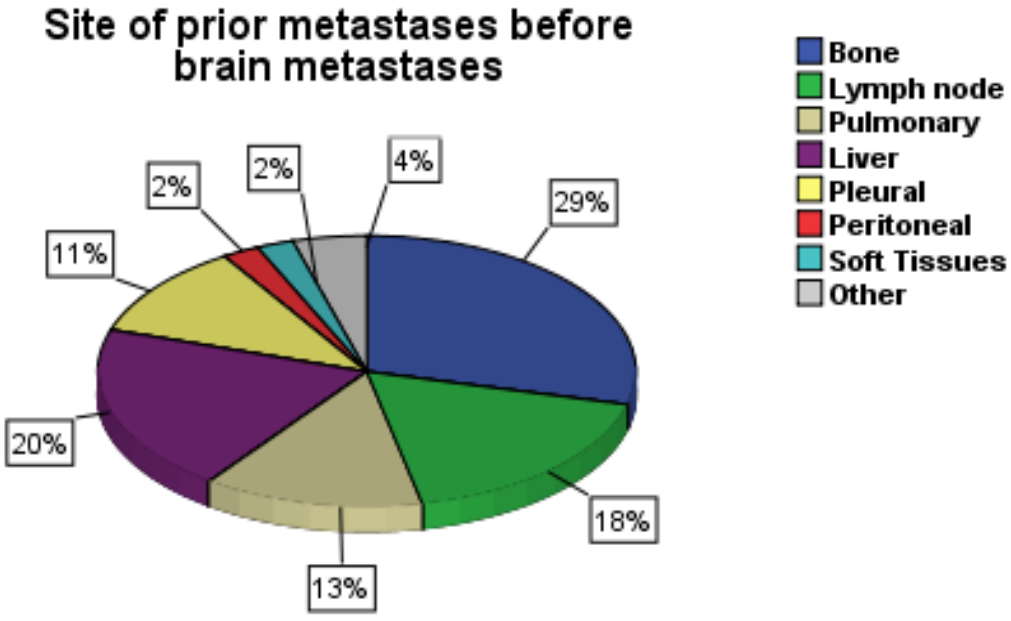

Figure 8- Distribution of cases of site of other metastases at diagnosis of brain metastases (%, relatively all metastases N=15).

At diagnosis of CNS metastasis, 27% of patients were under chemotherapy (Figure 9), 48% under hormonal therapy (Figure 10) and 61% under anti-HER2 therapy (Figure 11).

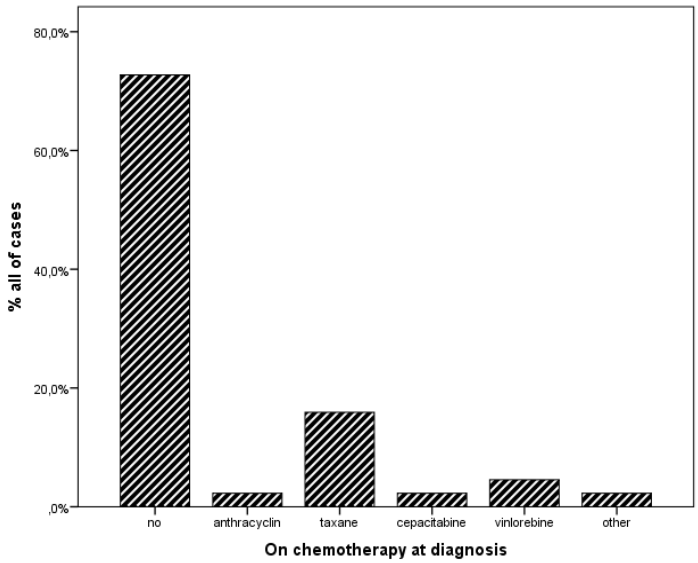

Figure 9- Chemotherapy regimens were being held at the moment of diagnosis brain metastases.

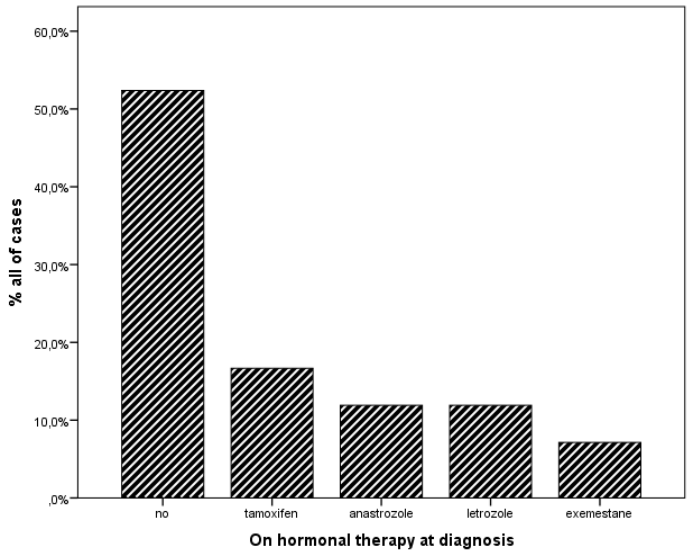

Figure 10- Hormonal therapy regimens were being held at the moment of diagnosis brain metastases.

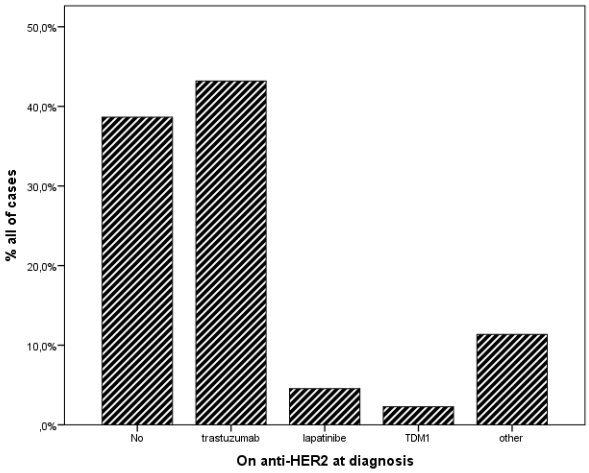

Figure 11- Anti- HER2 regimens were being held at the moment of diagnosis brain metastases.

After BM metastases diagnosis, most patients (57%) underwent whole brain radiotherapy (WBRT) only. Neurosurgery or radiosurgery followed by WBRT was performed in 18% of patients (Figure 12). Less than half of patients underwent further treatment with chemotherapy (49%) or hormonal therapy (44%) after local treatment (Figures 13 and 14). Systemic treatment without prior local treatment was performed in only 7% of patients. Trastuzumab was offered to 40% of patients, lapatinib to 34.9% and pertuzumab to 2.3% (Figure 15). Best supportive care was offered to 13.6% of patients.

In the last month before death, one patient had neurosurgery, 2 patients were on radiotherapy, and most patients (75%) were off any systemic treatment and under best supportive care.

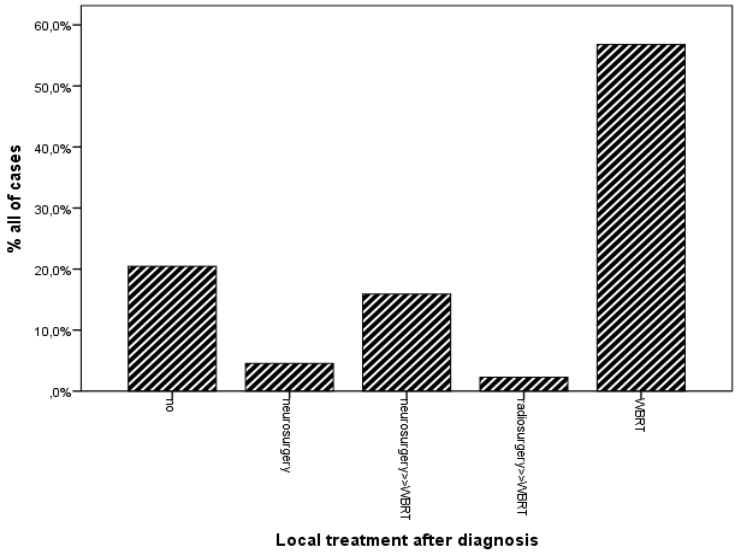

Figure 12- Local treatment after diagnosis of CNS metastases.

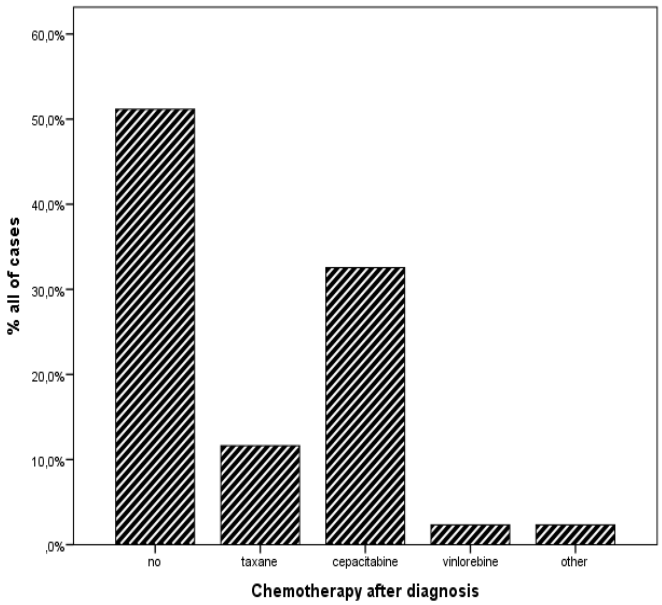

Figure 14-Chemotherapy performed after diagnosis after diagnosis of CNS metastases.

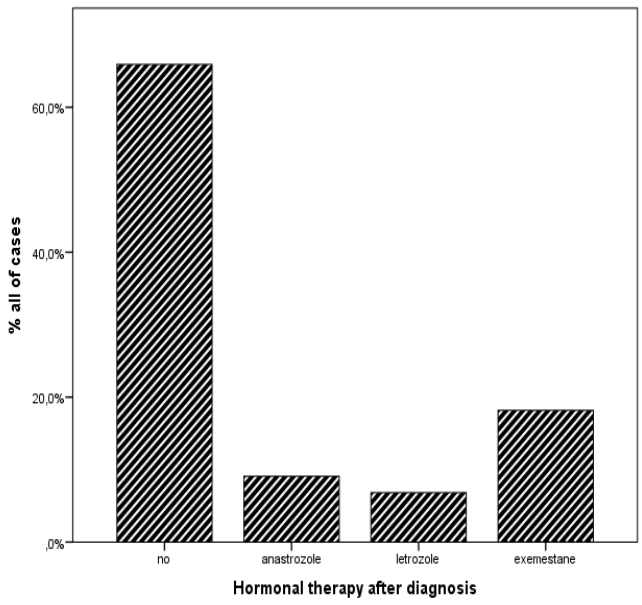

Figure 13-Hormonal therapy performed after diagnosis after diagnosis of CNS metastases.

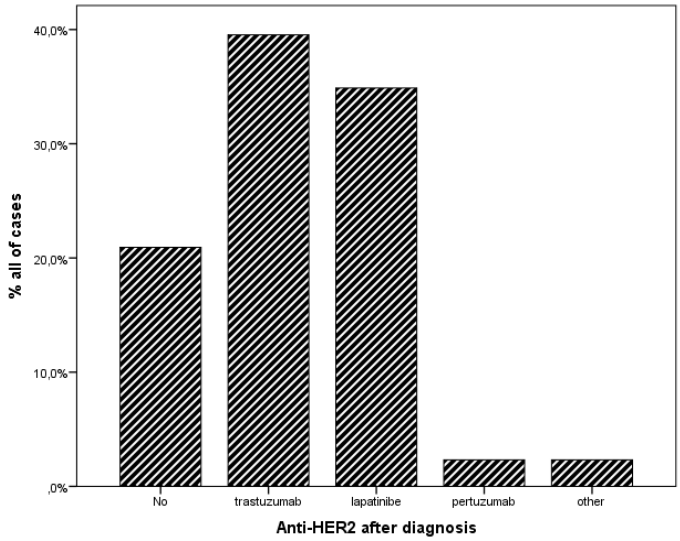

Figure 15- Anti-HER2 therapy after diagnosis after diagnosis of CNS metastases.

III – OUTCOME ANALYSIS

BRAIN METASTASES-FREE SURVIVAL

Median brain metastases-free survival (BMFS) in HER2+ BC patients was 25.5 months (95% IC 21.8-29.2). Figure 16 shows global median BMFS and Table V depicts the prognostically significant clinicopathological variables in HER2+ BC patients with BM, in univariate analysis.

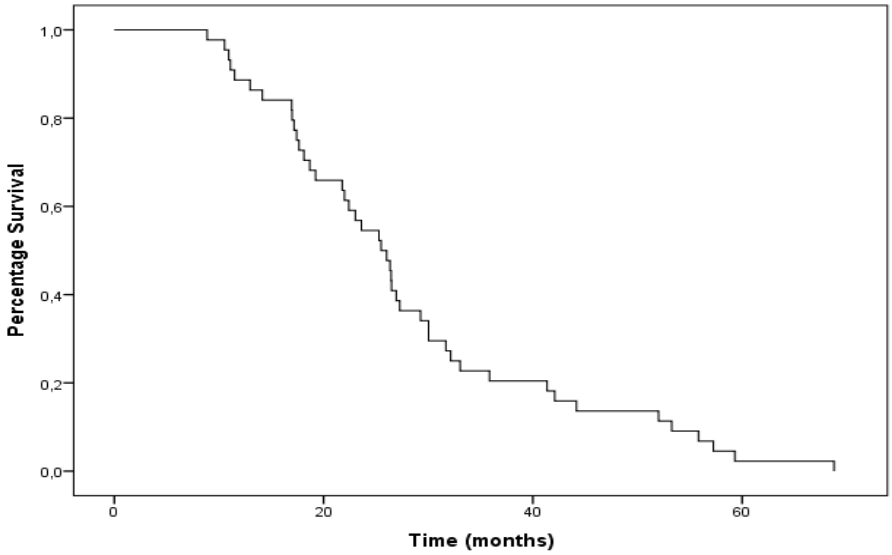

Figure 16- Brain metastasis-free survival of patients HER2-positive breast cancer with brain metastases.

Table V- Prognostic significance of clinicopathological variables in HER2+BC patients for BM.

Clinicopathological Variable	No. of cases	HR	95% CI of ratio	p
Ca15.3<25 or Ca15.3>25	16vs28	0.32	0.7-2.6	0.302
Tumor Size <2 or >2	2vs42	1.69	0.7-40.4	0.100
No. lymph node <3 or >3	10vs34	0.58	0.9-3.8	0.126
TNM 0-III or TNM IV	22vs7	0.68	0.8-4.8	0.134
Bone metastases No or Yes	37vs7	0.34	0.6-3.2	0.421
Lymph node metastases No or Yes	42vs2	0.03	0.2-4.3	0.965
Liver metastases No or Yes	40vs4	0.37	0.5-4.1	0.484

Figures 17-25 describe BMFS in above clinicopathological variables. There was a trend for a lower BMFS in patients with value of Ca15.3>25; tumor size>2cm; lymph nodes>3; TNM IV; with bone, lymph node or liver metastases at diagnosis of breast cancer, with no statistically significance difference.

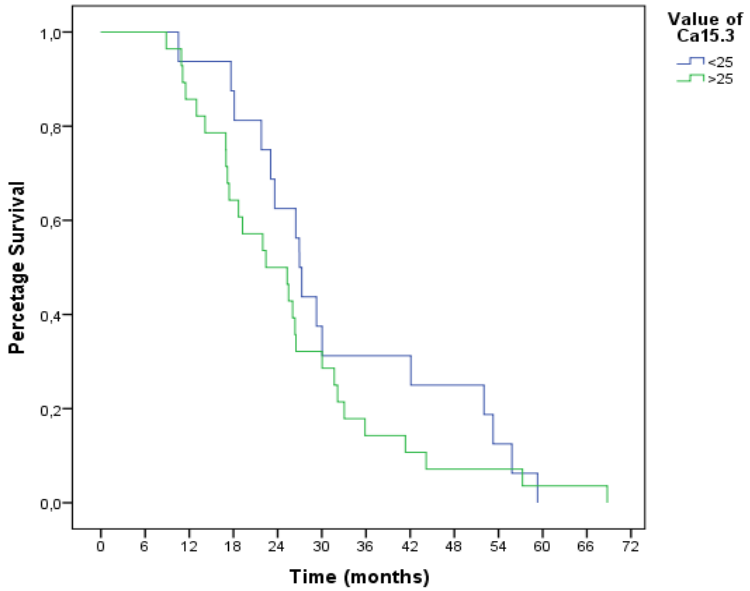

Figure 17- Brain metastases free survival in patients with value of Ca15.3 (<30 or >30) at diagnosis of HER2+BC patients in group with brain metastases. Median BMFS in patients with value of Ca15.3 <30 was 27 months and in patients with value >30 was 22 months (p=0.089).

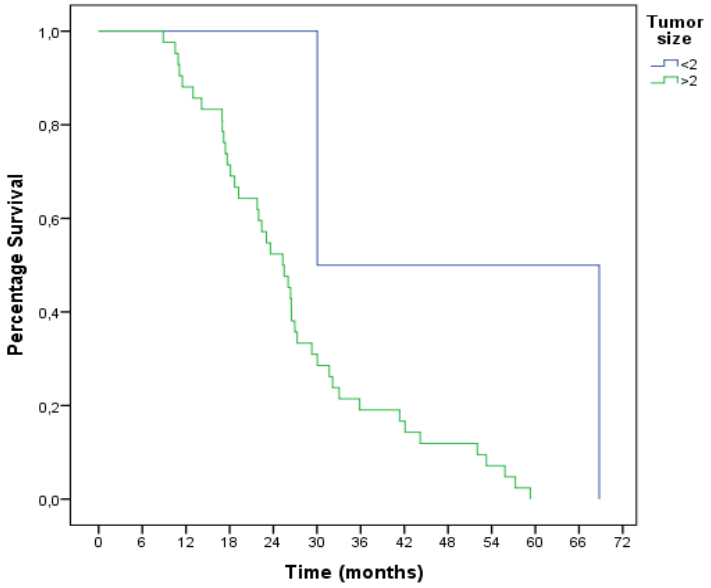

Figure 18- Brain metastases free survival in patients with tumor size <2 or >2 at diagnosis of HER2+BC patients in group with brain metastases. Median BMFS in patients with tumor size <2 was 30 months and in patients with tumor size >2 was 25 months (p=0.100).

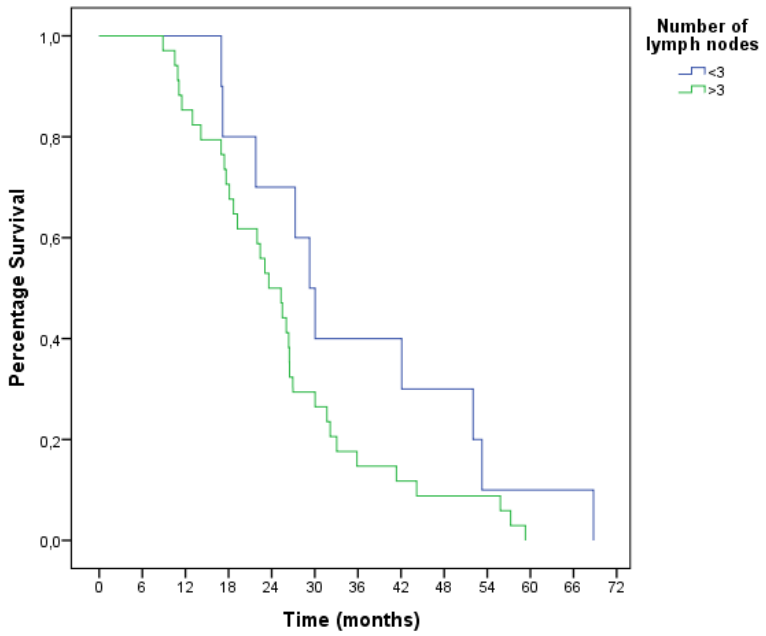

Figure 20- Brain metastases free survival in patients with number of lymph nodes <3 or >3 at diagnosis of HER2+BC patients in group with brain metastases. Median BMFS in patients with number of lymph nodes <3 was 29 months and in patients with number of lymph node >3 was 24 months (p=0.126).

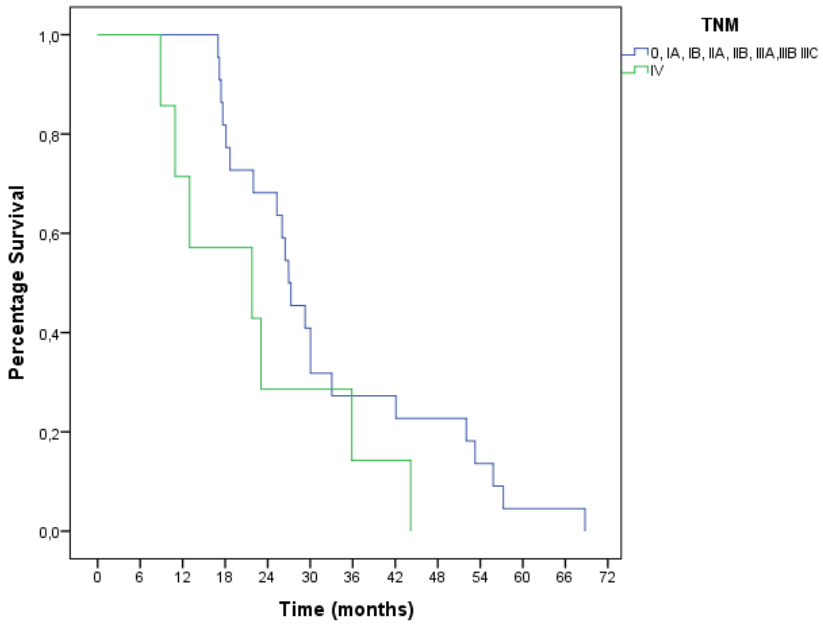

Figure 19- Brain metastases free survival in patients with TNM 0,I,II,III or TNM IV at diagnosis of HER2+BC patients in group with brain metastases. Median BMFS in patients with TNM 0,I,II,III was 27 months and in patients with TNM IV was 22 months (p=0.134).

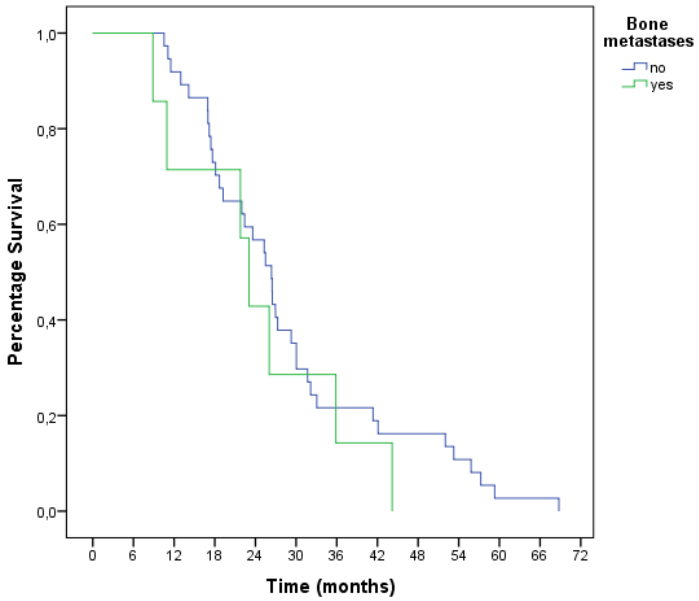

Figure 22- Brain metastases free survival in patients with or with no bone metastases at diagnosis of HER2+BC patients in group with brain metastases. Median BMFS in patients with no bone metastases was 26 months and in patients with bone metastases was 23 months (p=0.134).

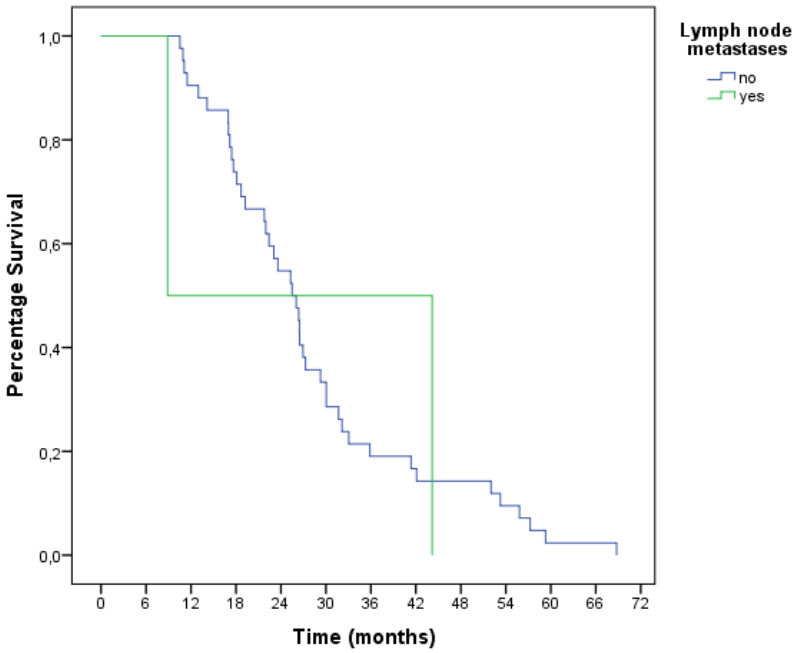

Figure 21- Brain metastases free survival in patients with or with no lymph node metastases at diagnosis of HER2+BC patients in group with brain metastases. Median BMFS in patients with no lymph node metastases was 25 months and in patients with lymph node metastase was 9 months (p=0.965).

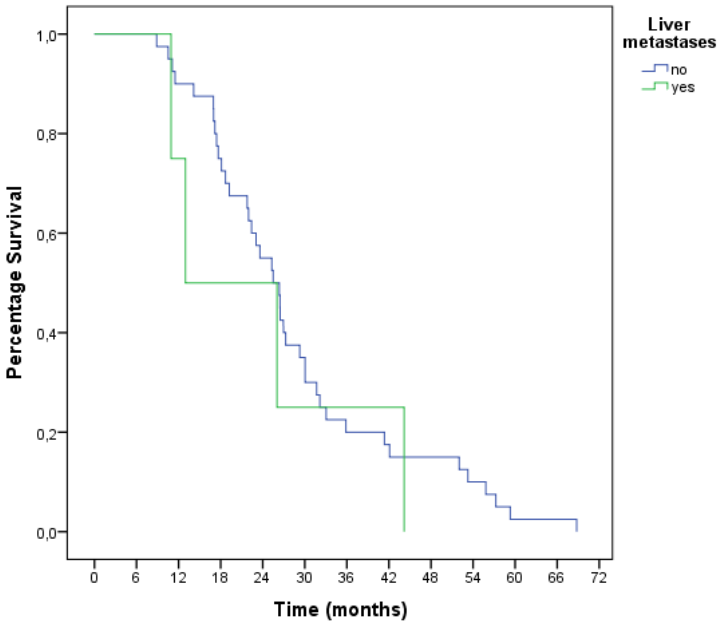

Figure 23- Brain metastases free survival in patients with or with no liver metastases at diagnosis of HER2+BC patients in group with brain metastases. Median BMFS in patients with no liver metastases was 25 months and in patients with liver metastase was 13 months (p=0.484).

OVERALL SURVIVAL

In HER2+ BC patients with BM, OS was 40.3±21.1 months and in HER2+BC patients with no BM metastases it was 52.2±8.4 months (p<0.05 95%, CI 0.8-12.2) (Figure 16).

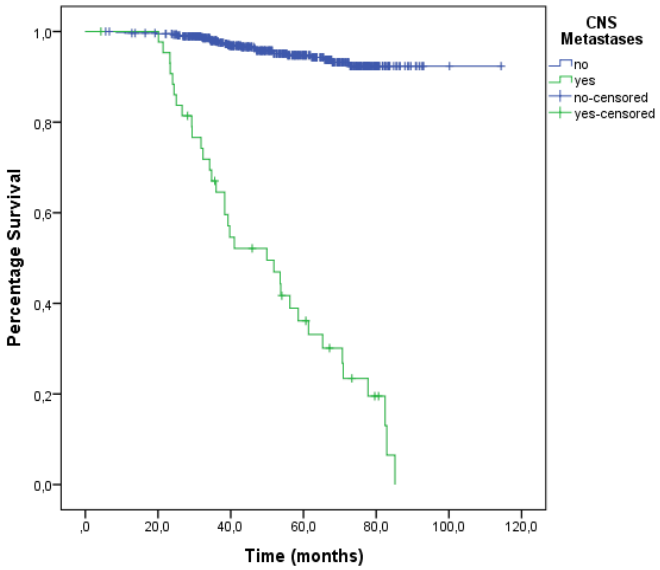

Figure 24- Overall survival in patients with HER2+ BC with no brain metastases (blue curve) and with brain metastases (green curve) (p<0.05).

SURVIVAL AFTER BRAIN METASTASIS

Median survival after developing BM in HER2-positive BC was 18 months (95% CI 12.6-23.4).

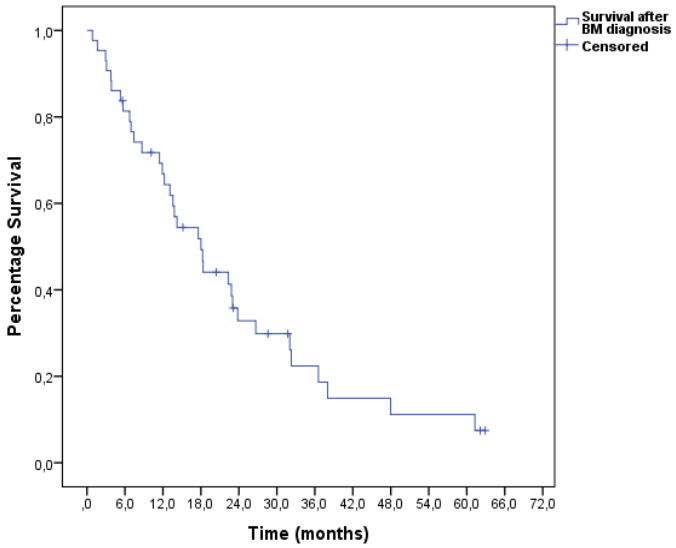

Figure 25- Median brain metastases free survival in patients with HER2+ BC with brain metastases.

DISCUSSION

In our study, incidence of BM metastases in HER2-positive BC was 7%, which is in agreement with a retrospective study of 9,524 HER2-positive BC patients[16]. Similarly, the median age of 51 years-old upon BM metastases diagnosis was similar to that of German, Japanese and Asian studies [20], [21], [22]. Nevertheless, we found that 94% of HER2-positive BC patients have symptoms upon BM diagnosis contrasting to the 39% reported in another study[18], a discrepancy which might be explained by the overuse of brain imaging in asymptomatic patients in that series.

Brain metastases as first site of disease relapse occurred in 61% of HER2-positive BC patients, most of them having more than 3 metastases (69%), similarly to other studies that revealed multiple BM as the rule[20] [22] [24]. Most common site of metastases were brain hemispheres (53%), cerebellum (37%), brainstem (7%) and meningeal (2%). The same order by frequency was found in other study, but with higher incidence in hemispheres (79%), lower in cerebellum (9%) and brain stem (2%)[20].

In the registHER study, 37.3% of patients with HER2-positive tumors displayed BM metastases, 7% at the time of their initial metastatic BC diagnosis and 30% as a subsequent site of disease[17]. In our study, 50% of HER2-positive BC patients were previously metastasized in other sites, namely bone, liver, lymph node, lung, pleural, brain, peritoneal and soft tissue. Those patients who were synchronously metastatic at BM diagnosis differed by order or frequency: lung, lymph node and pleura, bone, liver and meningeal metastases. Several studies showed that other sites of metastatic disease are similar [20][24]. Nevertheless those studies did not reported if metastases were previous or synchronous to BM.

We tried to identify risk factors for development of BM in HER2-positive BC. Younger age (<35 years), which is generally considered a risk factor for brain relapse in general BC population[25] was not demonstrated. Indeed, in our study the majority of cases peaked at 51 years. Also, hormonal status, prior pregnancy, personal/familial history of breast or ovarian cancer or body surface did not predict brain relapse. Nevertheless, this study demonstrates for the first time that performance status ECOG≥1 upon diagnosis of HER2-positive BC is associated with higher risk for development of brain metastases.

Furthermore, our data reveals that patients with larger tumors and node-positive disease had a significantly higher incidence of BM metastases, which is concordant with other published series in a general BC population[25][26]. However, higher tumor grade was not associated with BM, contrary to other studies[16]. Although HER2+/ER- BC is associated with higher risk of BM [26][24]., we did not find differences concerning hormonal receptor status. Additionally,

histological type, lymphovascular permeation or DCIS were not predictive of higher risk of BM.

Interestingly, we found that Ca15.3 was significantly more elevated at diagnosis in HER2-positive BC patients who developed BM. This finding is in line with the higher incidence of BM found in stage IV patients in this subpopulation, compared to earlier TNM stages at diagnosis. Bone, lymph node and liver metastases in HER2-positive BC patients upon diagnosis were also associated to a higher incidence of BM. This finding raises the hypothesis that CNS might represent a sanctuary for those patients that harbor disease controlled with initial systemic therapy, which does not usually penetrate the CNS[27].

After BM diagnosis, most patients underwent WBRT alone (57%) or neurosurgery/radiosurgery followed by WBRT (18%). The majority of patients received anti-HER2 therapy (77%). Almost half of the patients underwent chemotherapy (49%) or hormonal therapy (44%) as first systemic treatment after BM. In a clinical study, radiotherapy (93%) and chemotherapy (57%) were the most common treatments after BM diagnosis. Nevertheless anti-HER2 and neurosurgery (13%) were offered in the same proportion as in our study[22].

Importantly, we found that in our series, most patients with HER2-positive BC with BM were off any local or systemic anti-cancer therapy in the last month of life. Furthermore 75% of patients were already under best supportive care. Thus, the majority of those patients received the most adequate support, in line with good clinical practice[28].

In one study, causes of death included BM progression (35%) and systemic disease progression (30%), without specification in 35% of patients[20]. Other studies revealed that 55% of patients died due to complications of BM [22]. In our study, 86% of HER2-positive BC patients with BM died with evidence of cancer but it was not possible to determine if it was due to BM or systemic disease progression.

Median BMFS in HER2-positive BC patients was 25.5 months, which was similar to other published studies[22][29]. Overall survival in HER2+ BC patients with BM metastases was 40.3 months, which was significantly inferior to patients who do not develop BM (52 months) and is in line with other series [20][18]. Moreover, median survival after developing BM (SABM) in HER2-positive BC patients (18 months) is superior to other studies, that report SABM of about 9 months[21]. Poorest survival in these older studies is probably explained by the absence of trastuzumab use. Nevertheless, 18 months of SABM is similar to that reported for patients that undergo trastuzumab treatment, like in our series[22].

CONCLUSION

Incidence of BM in HER2+ BC patients in this Portuguese population was similar to other studies. Our study showed that some baseline clinical-pathological parameters might predict for BM development in HER2+ BC patients. Indeed, worse performance status and higher Ca15.3 value upon BC diagnosis were associated, for the first time, with an increased risk of developing BM,. However, additional studies, especially those of prospective design, are needed to validate these emerging data. Median survival after developing BM was longer than in previous studies, probably due to trastuzumab use in our patients. Nevertheless, overall survival in HER2+ BC patients with CNS metastases was shorter than in patients that did not have BM and additional research is needed to identify more effective treatment regimens.

DISCLOSURE

The authors declare that they have no conflicts of interest. This an academic study without any financial support from the industry.

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