Routine clinical parameters and laboratory testing predict therapy-related myeloid neoplasms after treatment for breast cancer

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Abstract

We aim to identify predictors of therapy-related myeloid neoplasms (t-MN) in patients with breast cancer (BC) and cytopenias to determine the timing of bone marrow biopsy (BMBx). Patients with BC and cytopenias who were referred for BMBx between 2002-2018 were identified using the Memorial Sloan Kettering Cancer Center institutional database. Characteristics associated with the risk of t-MN were evaluated by multivariable logistic regression and included in a predictive model. The average area under the receiver operating characteristic curve (AUC) was estimated by 5-fold cross-validation. Of the 206 BC patients who underwent BMBx included in our study, 107 had t-MN. By multivariable analysis, white blood cell count 4-11 K/mcL, absolute neutrophil count (ANC) ≥1.5 K/mcL, hemoglobin ≥12.2 g/dL, red cell distribution width 11.5-14.5%, the presence of bone metastasis and a time from BC diagnosis to BMBx <15 months significantly decreased the likelihood of t-MN. The average AUC was 0.88. We stratified our cohort by bone metastasis and by findings on peripheral smear. In both the subset without bone metastasis (n=159) and in the cohort with no blasts or dysplastic cells on peripheral smear (n=96) our variables had similar effects on the risk of t-MN. Among the 47 patients with bone metastasis, an ANC ≥1.5 K/mcL was the only variable associated with a decreased risk of t-MN. Our findings show that in patients with BC and unexplained cytopenias, clinical and laboratory parameters can predict t-MN and assist clinicians in determining the timing of a BMBx.

Introduction

Therapy-related myelodysplastic syndrome (t-MDS) and therapy-related acute myeloid leukemia (t-AML) – collectively known as therapy-related myeloid neoplasms (t-MN) – are rare long-term complications of cytotoxic chemotherapy and radiotherapy for both solid tumors and hematologic malignancies (HM).[1] The incidence of t-MN is higher after exposure to DNA-damaging agents, especially alkylators, topoisomerase II-inhibitors, and radiation, all commonly used for the adjuvant and neo-adjuvant treatment of breast cancer (BC).[2,3] t-MN account for an estimated 10% to 20% of all myeloid neoplasms, and are associated with high risk cytogenetic abnormalities, conveying a poorer prognosis than *de novo* MDS and AML. As overall BC survival improved over the last several decades, the incidence of t-MN has increased.[2,4,5] t-MN occur at a rate of 0.6% to 1.8% after treatment for BC and the frequency increases between 5 and 10 years following initial BC diagnosis.[4,6-8] Given that there are currently more than 3.8 million women with a history of BC living in the United States, the burden of t-MN is significant.[9]

In routine clinical practice, a subset of patients presents to their breast oncologist with variable degrees of cytopenias months to years after completing adjuvant therapy. These cytopenias may be a result of prolonged myelosuppression from chemotherapy, newly metastatic BC infiltrating the bone marrow, or t-MN. We hypothesized that routine clinical and laboratory parameters in patients with BC and cytopenias can predict the presence of t-MN, and serve as a screening tool to evaluate which patients are most likely to have a t-MN.

Methods

Data were collected using the Memorial Sloan Kettering Cancer Center (MSKCC) database. Medical records were reviewed under a retrospective observational research protocol approved by the MSKCC Institutional Review Board, in accordance with the Declaration of Helsinki.

Variables

Patients with BC diagnosed between January 2002 and December 2018 who were referred to the hematology clinic for evaluation of unexplained cytopenias and underwent BMBx were included. Variables obtained comprised prior BC treatment, pre-existing bone metastasis, white blood cell (WBC) count, absolute neutrophil count (ANC), hemoglobin (Hgb) level, mean corpuscular volume (MCV), red cell distribution width (RDW), platelet (PLT) count, peripheral smear at the time of BMBx, and BMBx findings. The diagnosis of t-MN was determined by MSKCC pathology reports that were categorized using the International Working Group criteria for MDS and AML. BC treatment was divided in categories: chemotherapy with radiotherapy, only chemotherapy, only radiotherapy, only surgery. Pancytopenia was defined as WBC <4 K/mcL, Hgb <12.2 g/dL and PLT <100 K/mcL. We used an ANC cutoff of <1.5 K/mcL, corresponding to grade 1 severity of adverse effects per the CTCAE, and followed the National Cancer Institute's criteria for anemia in women.[10] We adopted the MSKCC laboratory normal range for WBC (4-11 K/mcL), MCV (82-98 fL) and RDW (11.5-14.5%). The follow-up period was heterogeneous as it was defined by the interval from referral and chart review. Clinically relevant characteristics and laboratory parameters were selected based on their reported association with t-MN [4,11-13] and assessed as predictors of t-MN.

Statistical analysis

Multivariable logistic regression with both-directional stepwise selection was used to determine the best predictors of t-MN. Starting model contained intercept and a variable leading to the smallest Akaike information criterion (AIC) was selected at each step of the algorithm. Final models that minimized AIC were selected.

In order to explore associations between patient characteristics and t-MN, we defined the multiplicative factor for the patient i as $R_i=exp(\sum^{p}_{j=1} \hat{\beta}_j X_{ij})$, where X_{ij} are selected characteristics by the final model, and $\hat{\beta}_j$ are estimated log-odds ratios. The ratio of multiplicative factor R_i calculated for risk factors $(X_{i1},...,X_{ip})$ to a multiplicative factor R_k calculated for risk factors $(X_{k1},...,X_{kp})$, represents the change in odds of a patient i having t-MN compared to a patient k. Log-values of R_i called risk scores, $S_i=\sum^{p}_{j=1} \hat{\beta}_j X_{ij}$ were calculated for each patient using the final model. For this scoring system, each variable was assigned a score by rounding $\hat{\beta}_j$ to nearest integer.

The average area under the receiver operating characteristic curve (AUC) of the final predictive model was estimated by 5-fold cross-validation. In each round of cross-validation, we applied the multivariable logistic regression with the same stepwise selection to training data and estimated AUC on test data. The population was divided by bone metastasis and by results of peripheral smear. The multivariable logistic regression with stepwise selection was applied to determine the best predictors of t-MN on the non-bone metastatic cohort (n=159) and on the smear negative cohort (n=96); univariate analysis was performed on the much smaller bone-metastatic subset (n=47).

Results

A total of 359 patients with prior treatment for BC were referred to the MSKCC hematology clinic for the evaluation of cytopenias and underwent a BMBx. One hundred and fifty-three patients were excluded from the study, primarily due to a prior diagnosis of a hematologic malignancy or another solid tumor preceding their BC diagnosis, or if they had a diagnosis of a myeloproliferative neoplasm, lymphoma or multiple myeloma on the subsequent BMBx results (see CONSORT diagram). A total of 206 patients were included in the final analysis (Table 1).

Analysis of the whole cohort

Of the 206 patients, 137 received chemotherapy; the majority were exposed to an alkylating agent (58.7%) and to an agent targeting DNA-topoisomerase II (49.5%). A total of 142 patients received RT and 117 had hormone treatment (see *Online Supplementary Tables S1, S2* and *S3*). A total of 107 had BMBx findings consistent with a t-MN (crude incidence of 51%). Specifically, 47 patients were diagnosed with t-AML, 44 patients had t-MDS, and 16 patients had an initial diagnosis of t-MDS that evolved to t-AML during the follow-up period. Forty-five patients had BMBx findings of metastatic carcinoma compatible with a BC primary. One hundred and twenty-one patients died

Table 1. Characteristics of the whole population.

Characteristics	All pts N=206	Pts without bone mets N=159	Pts with bone mets N=47
Race, N (%)			
Asian	8 (3.8)	8 (5)	0 (0)
Black	36 (17)	28 (17)	8 (17)
White	152 (73.7)	114 (71.7)	38 (80.8)
Unknown	10 (4)	9 (5.7)	1 (2)
Age in years at BC Dx			
Median age (IQR)	56 (18)	57 (16)	53 (22.5)
Age in years at BMBx			
Median age (IQR)	64.5 (17)	65 (16.5)	61 (23.5)
Latency between BC Dx and BMBx			
Median years (IQR)	5 (7)	5 (7)	4 (6)
BMBx findings, N (%)			
t-AML	47 (22.8)	45 (28)	2 (4)
t-MDS	44 (21)	43 (27)	1 (2)
t-MDS > t-AML	16 (7.7)	14 (8.8)	2 (4)
Bone mets	45 (21.8)	8 (5)	37 (78)
No abnormalities	54 (26)	47 (29.5)	5 (10)

Pts: patients; mets: metastasis; BC: breast cancer; Dx: diagnosis; BMBx: bone marrow biopsy; t-MDS: therapy-related myelodysplastic syndrome; t-AML: therapy-related acute myeloid leukemia; IQR: interquartile range.

during the study period and 17 were lost to follow-up. We evaluated the cytogenetics of patients diagnosed with t-MN and found that 33 of them had a normal karyotype, 35 had a single chromosomal abnormality, 37 presented with multiple chromosomal aberrations and two had unknown cytogenetics. Similar to prior studies,[14-16] the most common abnormalities were deletion or loss of chromosomes 5 and 7 (21.5% and 13%, respectively), followed by trisomy of chromosome 8 and t(11q23), (13% and 9%, respectively). As shown in the *Online Supplementary Table S4*, we observed that the use of alkylating agents was more frequently associated with deletions or loss of chromosomes 5 and 7, while treatment with agents targeting DNA-topoisomerase II was associated with balanced translocations such as t(8;21) and t(11q23). In the univariate analysis of the total cohort, we observed nine clinical variables with *P* values below 0.05 (see the *Online Supplementary Table S1*). In particular, a WBC 4-11 K/mcL ($P<0.0001$), an ANC ≥1.5 K/mcL ($P<0.0001$), a Hgb level ≥12.2 g/dL ($P=0.0009$), a MCV <98 ($P=0.0001$), a normal RDW ($P= 0.0001$), a PLT count ≥100 K/mcL ($P=0.0075$), the absence of dysplastic cells or blasts in the peripheral smear ($P<0.0001$), the presence of known bone metastasis ($P<0.0001$) and a time from BC diagnosis to BMBx of <15 months ($P<0.0001$) significantly decreased the likelihood of t-MN. No relationship was associated with race ($P=0.27$) and with a history of chemotherapy or radiotherapy ($P=0.12$). When we created our prediction model, we excluded the peripheral smear because 49 of 52 patients (94%) with blasts or dysplastic cells had a diagnosis of t-MN and only three patients (6%) had a positive smear without t-MN. Our final multivariable logistic model included WBC, ANC, Hgb, RDW, the presence of bone metastasis and a time from BC diagnosis to BMBx <15 months (Table 2). Figure 1A shows the distribution of risk scores for being diagnosed with t-MN in the whole cohort. The score ranges from -11 to 4, with score increase implying a higher risk of having t-MN. Patients diagnosed with t-MN had risk scores ranging between -5 and 4 and non-t-MN patients had risk scores ranging between -11 and 1. A *post hoc* value of -5 can be used as a cutoff and corresponds to 100% true positive rate and acceptable false positive rate of 50%, at our institution. The score also showed good discrimination between the two groups resulting in an average AUC of 0.88 (95% confidence interval [CI]: 0.85-0.91) (Figure 2A).

Analysis of the cohort stratified by bone metastasis

The cohort was stratified by the presence of bone metastasis. A total of 159 patients did not have pre-existing bone metastasis and, in this subset, 102 patients were diagnosed with t-MN. Out of this group, 43 patients had t-MDS, 45 patients were found to have t-AML, and 14 patients had initially t-MDS that evolved to t-AML during the follow-up period; finally, eight patients had BMBx findings revealing metastatic BC. The univariate analysis of this group showed similar results as the whole cohort (*Online Supplementary Table 5S*). A WBC 4-11 K/mcL, an ANC ≥1.5 K/mcL, a Hgb level ≥12.2 g/dL, a MCV <98, a normal RDW, a PLT count ≥100 K/mcL, a normal peripheral smear, and a time from diagnosis to BMBx of <15 months were associated with a decreased probability of being di-

Table 2. Associations with the clinical characteristics selected for the final model based on the whole cohort.

Variable	All pts N=206	Pts with t-MN N=107 (%)	Pts without t-MN N=99 (%)	OR (95% CI)*	*P*	Score
WBC					0.0003	
4-11 x 10^9/L	87	29 (33)	58 (67)	1		0
<4 x 10^9/L	87	53 (61)	34 (39)	0.41 (0.09-1.76)		-1
>11 x 10^9/L	31	24 (77)	7 (23)	10.91 (2.46-48.36)		2
ANC					0.0002	
<1.5 x 10^9/L	66	52 (79)	14 (21)	1		0
≥1.5 x 10^9/L	127	43 (34)	84 (66)	0.05 (0.01-0.23)		-3
Hgb					0.0036	
<12.2 g/dL	163	94 (58)	69 (42)	1		0
≥12.2 g/dL	42	12 (29)	30 (71)	0.14 (0.04-0.53)		-2
RDW					0.038	
11.5-14.5%	51	12 (24)	39 (76)	1		0
>14.5%	131	74 (56)	57 (44)	3.1 (1.07-8.99)		1
Bone mets					<0.0001	
Absent	159	102 (64)	57 (36)	1		0
Present	47	5 (11)	42 (89)	0.03 (0.01-0.11)		-4
Time from BC Dx to BMBx					0.0031	
>15 months	166	97 (58)	69 (42)	1		0
<15 months	38	8 (21)	30 (79)	0.16 (0.05-0.54)		-2

*Estimates from multivariable logistic regression with selected predictors in the model. Pts: patients; t-MN: therapy-related myeloid neoplasms; OR: odds ratio; CI: confidence interval; WBC: white blood cell; ANC: absolute neutrophil count; Hgb: hemoglobin; RDW: red cell distribution width; mets: metastasis; BC: breast cancer; Dx: diagnosis; BMBx: bone marrow biopsy.

agnosed with t-MN. Our final multivariable logistic model was also comparable to the final model selected in the whole cohort (Table 3). The scoring system obtained with the final statistically significant variables is shown in Figure 1B. The distribution ranges from -6 to 5 with higher scores conveying an increased risk of having t-MN. Patients with t-MN had a risk score ranging between -6 and 2 while non-t-MN patients had a score between -4 and 4. It was difficult to identify a cutoff in this cohort since there was a true positive rate of 100% and a high false positive rate of 80%, at our institution. The score showed good discrimination between the two groups resulting in an average AUC of 0.81 (95% CI: 0.72-0.9), Figure 2B.

The subset with pre-existing bone metastasis consisted of 47 patients; five of them had a diagnosis of t-MN. The majority of the patients (78%, 37/47 patients) had BMBx findings consistent with metastatic BC. t-MDS was found in one patient, two had a diagnosis of t-AML and two patients had t-MDS that evolved to t-AML. In our univariate analysis (*Online Supplementary Table S6*), only an ANC ≥1.5 K/mcL was borderline significant (*P*=0.053). No risk score was calculated in this cohort.

Analysis of the cohort stratified by peripheral smear

Our cohort was stratified by the findings on the peripheral smear performed at the time of BMBx. We found 148 reports and 52 of them were positive for blasts or dysplastic cells. The majority of patients with a positive smear had a diagnosis of t-MN (94%, *P*=0.0001), and only three patients had BC bone metastasis instead. Since a positive smear was highly predictive of t-MN, we did not create a predictive model in this cohort.

Among the 96 patients with negative smear, 61% did not have a diagnosis of t-MN and 95% of patients without t-MN had a normal peripheral smear (*P*=<0.0001). In the univariate analysis of this group, a WBC 4-11 K/mcL, an ANC ≥1.5 K/mcL, a Hgb level ≥12.2 g/dL, a MCV <98, the presence of known bone metastasis and a time from diagnosis to BMBx of <15 months were correlated with a decreased probability of being diagnosed with t-MN (*Online Supplementary Table 7S*). Table 4 illustrates the final variables selected by multivariable analysis and Figure 1C shows the score system obtained with these variables. The distribution ranges from -9 to 3; similarly to the whole cohort, increase in scores conveyed a higher risk of having t-MN. Patients diagnosed with t-MN had risk scores ranging between -3 and 3 and non-t-MN patients had risk scores ranging between -9 and 3. A *post hoc* value of -3 can be used as a cutoff and corresponds to 100% true positive rate and acceptable false positive rate of 59%, at our institution. The score also showed good discrimination between the two groups resulting in an average AUC of 0.81 (95% CI: 0.69-0.92), see Figure 2C.

Discussion

As the overall survival of BC improves, the incidence of t-

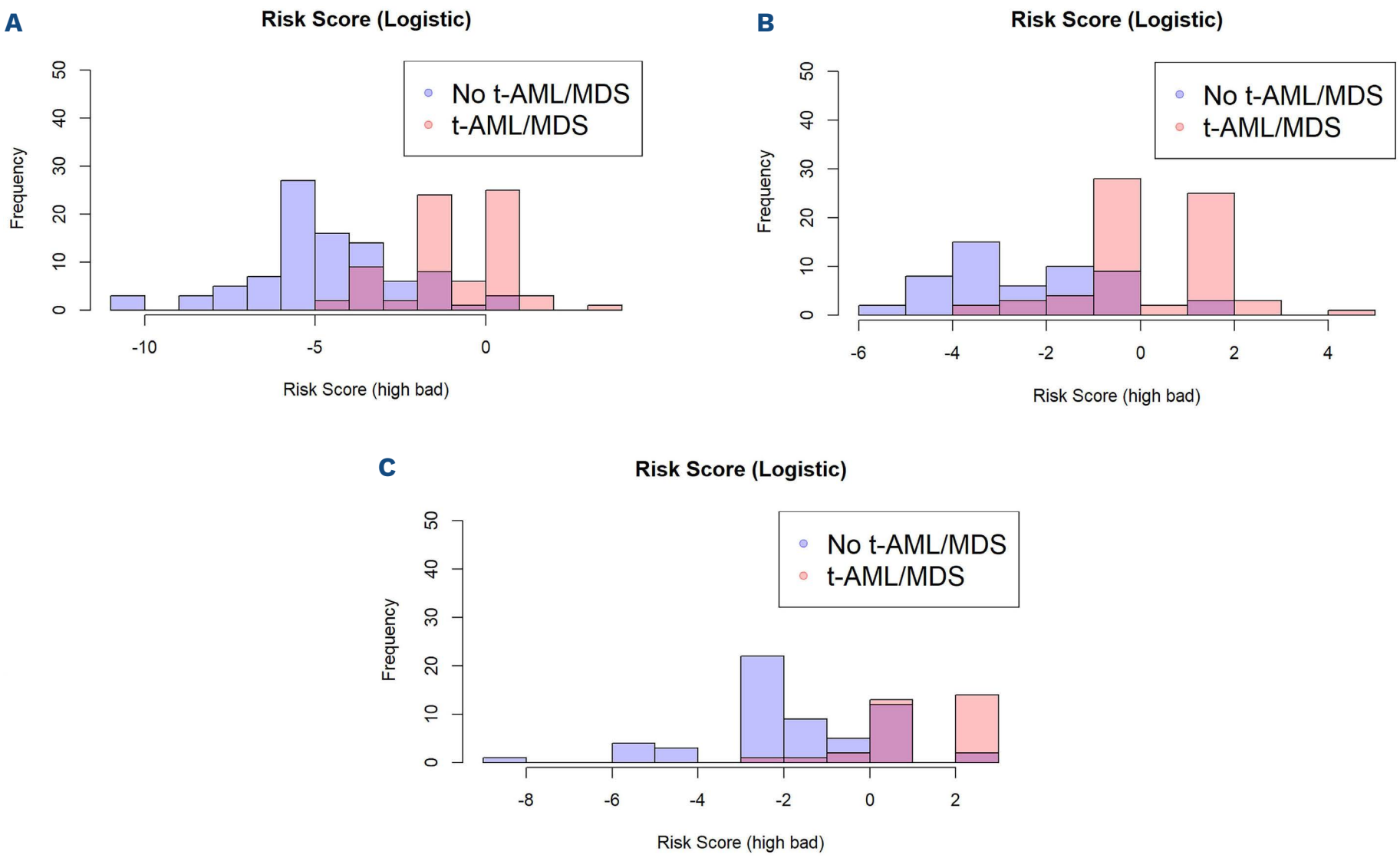

Figure 1. Scoring system obtained integrating the score assigned to each statistically significant variable to help determine a probability of having therapy-related myeloid neoplasms. (A) Distribution of risk scores in the whole cohort (n=206) for the probability of being diagnosed with therapy-related myeloid neoplasms (t-MN). The blue columns represent patients without t-MN, the red ones represent patients with t-MN and the purple columns show the overlap between the 2 cohorts. The score ranges from -11 to 4, with higher scores conveying a greater risk of having t-MN. Patients diagnosed with t-MN had risk scores ranging between -5 and 4 and non-t-MN patients had risk scores ranging between -11 and 1. A *post hoc* value of -5 can be used as a cutoff and corresponds to 100% true positive rate and acceptable false positive rate of 50%; at our institution. (B) Distribution of risk scores in the cohort without bone metastasis (n=159) for the probability of being diagnosed with t-MN. The distribution ranges from -6 to 5 with higher scores conveying an increased risk of having t-MN. Patients with t-MN had a risk score ranging between -6 and 2, while non-t-MN patients had a score between -4 and 4. It was difficult to identify a cutoff in this cohort since there was a true positive rate of 100% and a high false positive rate of 80%; at our institution. (C) Distribution of risk scores in the cohort with negative peripheral smear (n=96) for the probability of being diagnosed with t-MN. The distribution ranges from -9 to 3; increase in scores conveyed a higher risk of having t-MN. Patients diagnosed with t-MN had risk scores ranging between -3 and 3 and non-t-MN patients had risk scores ranging between -9 and 3. A *post hoc* value of -3 can be used as a cutoff and corresponds to 100% true positive rate and acceptable false positive rate of 59%; at our institution.

MN after treatment for BC continues to rise. In this study, we sought to identify predictors of t-MN in order to assist in the early diagnosis of t-MN and determine the best timing of BMBx in patients with new unexplained cytopenias and prior treatment for BC. It is known that specific factors are more often correlated with a higher risk of developing t-MN, especially high doses of chemotherapy, long duration of treatment, combination with radiotherapy and older age.[17] However, because of the very complex nature of t-MN and of the acuity of t-AML, formulating predictive models can be challenging. Prior studies have tried to create predictive models for the early diagnosis of t-MN using clonal hematopoiesis.[18,19] Both Genovese and Takahashi[20,21] found that age-related clonal hematopoiesis (ARCH) could be used as a biomarker for prediction of AML because the presence of a myeloid clone, detected after treatment for the primary cancer, would increase the possibility of subsequent t-MN. Nevertheless, to use ARCH as a predictive tool, a large number of patients need to be tested incurring substantial costs. A good biomarker should be objectively measured in a non-invasive fashion, it should be easy to obtain and inexpensive, in order to be used repeatedly and applied to a large population.[22] To our knowledge, this is the first study that uses only routine clinical and laboratory parameters as predictors of t-MN. When we analyzed the CBC obtained at the same time as the BMBx, we found a statistically significant association with both the WBC and the ANC and the probability of having

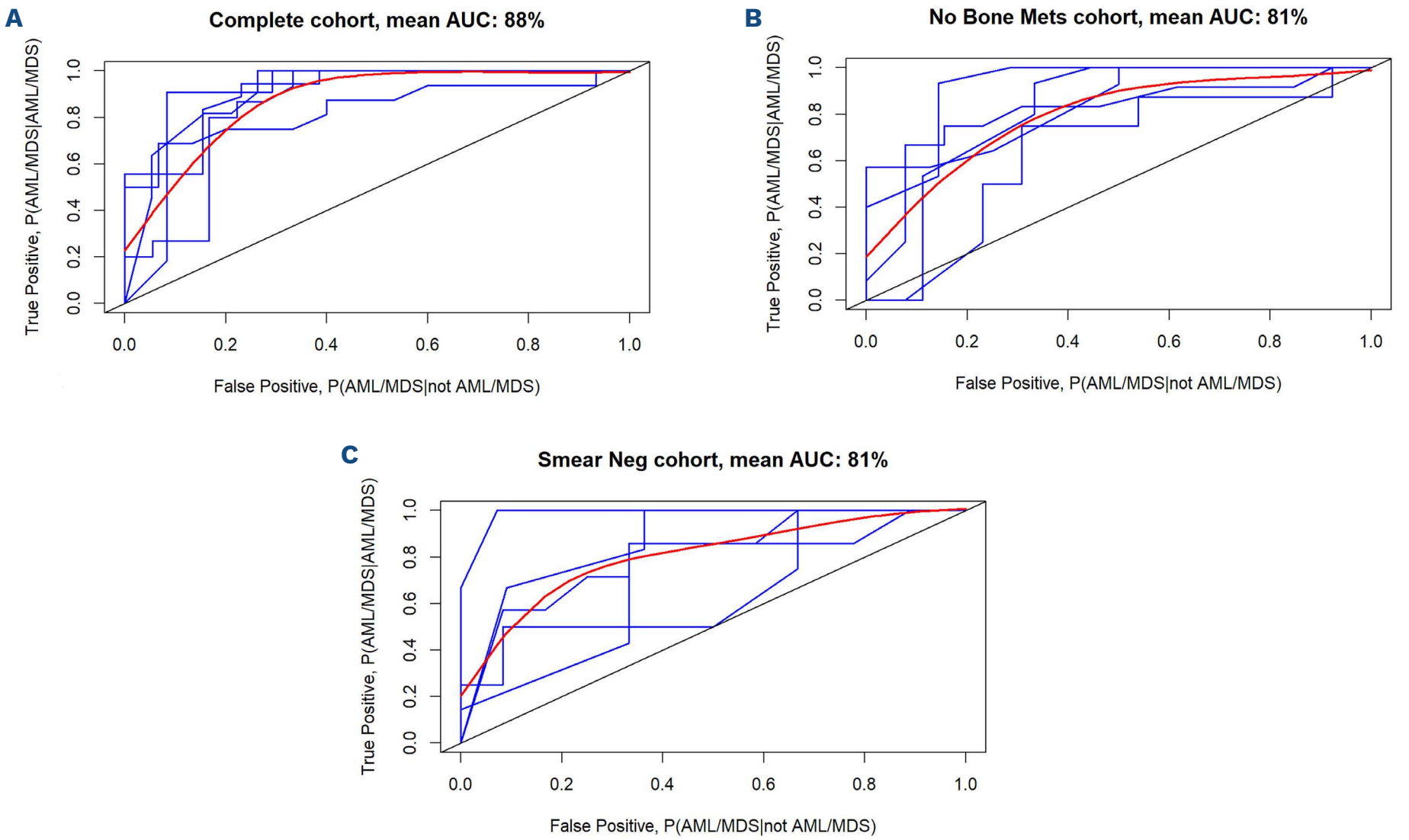

Figure 2. Average receiver operating characteristic curves of the logistic model selected by both-directional stepwise logistic regression. Blue receiver operating characteristic (ROC) curve is estimated at each run of 5-fold cross-validation. Red ROC curve represents an average ROC curve obtained by fitting a smooth curve to the estimated blue ROC curves. (A) Average ROC curve for the whole cohort. (B) Average ROC curve for the subset without bone metastasis. (C) Average ROC curve for the subset with negative peripheral smear. AUC: average area under the receiver operating characteristic curve; AML: acute myeloid leukemia; MDS: myelodysplastic syndrome; Neg: negative; Mets: metastasis.

Table 3. Associations with the clinical characteristics selected for the final model based on the cohort without bone metastasis.

Variable	Pts without bone mets N=159	Pts with t-MN N=102 (%)	Pts without t-MN N=57 (%)	OR (95% CI)*	*P*	Score
WBC					0.004	
<4 x 10^9/L	75	50 (67)	25 (33)	1		0
>11 x 10^9/L	25	22 (88)	3 (12)	23.21 (2.71-199)		3
4-11 x 10^9/L	58	29 (50)	29 (50)	2.67 (0.61-11.8)		1
ANC					0.0007	
<1.5 x 10^9/L	62	50 (81)	12 (19)	1		0
≥1.5 x 10^9/L	85	40 (47)	45 (53)	0.06 (0.01-0.31)		-3
Hgb					0.0051	
<12.2 g/dL	124	89 (72)	35 (28)	1		0
≥12.2 g/dL	34	12 (35)	22 (65)	0.16 (0.05-0.58)		-2
RDW					0.034	
11.5-14.5%	45	12 (27)	33 (73)	1		0
>14.5%	92	69 (75)	23 (25)	3.07 (1.09-8.65)		1
Time from BC Dx to BMBx					0.0057	
>15 months	129	92 (71)	37 (29)	1		0
<15 months	28	8 (29)	20 (71)	0.19 (0.06-0.61)		-2

*Estimates from multivariable logistic regression with selected predictors in the model. mets: metastasis; t-MN: therapy-related myeloid neoplasms; OR: odds ratio; CI: confidence interval; WBC: white blood cell; ANC: absolute neutrophil count; Hgb: hemoglobin; RDW: red cell distribution width; BC: breast cancer; Dx: diagnosis; BMBx: bone marrow biopsy.

a diagnosis of t-MN. In the analysis of the whole cohort, 58% and 85% of the patients without t-MN had a normal WBC count and an ANC ≥1.5 K/mcL, respectively. Similar results were observed in the subset of patients without pre-existing bone metastasis, where 51% and 79% of patients without t-MN had a WBC 4-11 K/mcL and an ANC ≥1.5 K/mcL, respectively. In the group with pre-existing bone metastasis, 95% of the patients without t-MN had an ANC ≥1.5 K/mcL. In this cohort, we did not find any statistically significant association with the rest of the variables, possibly due to the extremely small number of patients in this subgroup. Moreover, even though the majority of patients had anemia (79%),[10] we observed that a Hgb ≥12.2 g/dL was more frequently associated with the absence of t-MN. Our findings differ from prior studies, in fact, both Jaiswal and Abelson[23,24] found that patients with ARCH and pre-AML mutations had normal blood counts. The only difference they observed was a significantly raised RDW in patients at higher risk of progression to AML. However, a low WBC, ANC and hematocrit have been associated with an increased risk of development of MDS and AML, but no specific cutoff had been used to help identify a predictive tool.[12,13,25] Finally, when we examined the MCV and RDW values, we found that 86% of the patients without t-MN had an MCV <98 and 76% of them had a normal RDW. These results echo the association between both macrocytosis and higher RDW and an increased probability of developing AML and MDS already reported in previous studies.[23,24,26,27] Our findings suggest that t-MN usually have subtle, but recognizable clinical manifestations that can be useful to identify patients at risk.

Prior works have demonstrated an association between thrombocytopenia and risk of t-MN.[12,13,28] Pagano[29] and Linassier[25] found that at onset of t-AML, the average PLT count was 30-35 K/mcL. We used a cutoff PLT count of ≥100 K/mcL to better capture pathological conditions,[30] and found a positive association with the likelihood of t-MN in the whole cohort and in those without pre-existing bone metastasis. The prevalence of thrombocytopenia in t-MN can be related to the damage of bone marrow stem cells after chemotherapy and, due to their short lifespan, a low platelet count can be an early indication of t-MN.

When we reviewed the peripheral smear of the whole cohort, we found that 95% of patients without t-MN had a normal smear. Similarly, in the cohort without bone metastasis, 100% of patients without t-MN had normal findings in the smear. Only three patients without t-MN had peripheral blasts and were diagnosed with metastatic BC. This could be explained by the presence of leukoerythroblastosis and carcinocythemia associated with metastatic BC.[31-33] Our study confirms that the presence of blasts and dysplastic cells in the peripheral smear of patients with t-MN is expected[34,35] and represents another inexpensive tool to aid in the early diagnosis of t-MN.

Consistent with previous studies, our results showed an association with the latency between the diagnosis of the two cancers. In both the whole cohort and the subcohorts of patients without pre-existing bone metastasis and with normal peripheral smear, we found a decreased preva-

Table 4. Associations with the clinical characteristics selected for the final model based on the cohort with negative peripheral smear.

Variable	Pts with negative smear N=96	Pts with t-MN N=37 (%)	Pts without t-MN N=59 (%)	OR (95% CI)*	*P*	Score
WBC					0.053	
4-11 x 10^9/L	44	9 (20)	35 (80)	1		0
<4 x 10^9/L	41	22 (54)	19 (46)	1.7 (0.34-8.43)		1
>11 x 10^9/L	10	5 (50)	5 (50)	12.63 (1.49-107.09)		3
ANC					0.060	
<1.5 x 10^9/L	26	18 (69)	8 (31)	1		0
≥1.5 x 10^9/L	68	17 (25)	51 (75)	0.19 (0.03-1.07)		-2
Hgb					0.009	
<12.2 g/dL	80	35 (44)	45 (56)	1		0
≥12.2 g/dL	15	1 (7)	14 (93)	0.04 (0-0.43)		-3
Time from BC Dx to BMBx					0.007	
<15 months	26	4 (15)	22 (85)	1		0
>15 months	70	33 (47)	37 (53)	7.78 (1.76-34.46)		2
Bone mets					0.001	
Absent	69	35 (51)	34 (49)	1		0
Present	27	2 (7)	25 (93)	0.04 (0.01-0.29)		-3

*Estimates from multivariable logistic regression with selected predictors in the model. Pts: patients; mets: metastasis; t-MN: therapy-related myeloid neoplasms; OR: odds ratio; CI: confidence interval; WBC: white blood cell; ANC: absolute neutrophil count; Hgb: hemoglobin; BC: breast cancer; Dx: diagnosis; BMBx: bone marrow biopsy.

lence of t-MN if the time from the diagnosis of BC was less than 15 months (79%, 71% and 84% of the patients, respectively), with an average latency between BC diagnosis and BMBx of 5 years. This is in line with the well-known time frame between treatment with both alkylating agents and topoisomerase II inhibitors and the risk of secondary leukemias. Similarly, prior works also found an increased incidence of t-AML between 12 months to 9 years following the treatment of BC that then declined after 10 years from the initial BC diagnosis.[36-38]

The carcinogenesis and increased risk for secondary malignancies associated with chemotherapy and radiotherapy are well known.[12,39-42] Unexpectedly, in our study population we did not find a relationship with chemotherapy and radiotherapy. A possible explanation is the decreased use of leukemogenic agents such as melphalan in our population,[43-45] but it might also be secondary to other factors including the small population studied and the follow-up duration.

When we analyzed the association between race and risk of developing t-MN, there was no statistical significance. It needs to be specified that, due to small numbers of White Hispanic and Native American patients, they were included in the White race group. Nevertheless, similar data was discussed in a recent study on racial differences in secondary cancers that revealed a comparable elevated possibility of t-AML in BC patients among all races, with higher risk in Black and Asian/Pacific Islander women.[46] Wei *et al.*[38] observed an increased risk of secondary AML in White patients compared to other races. These findings highlight the need for further investigation on how genetic differences can influence the incidence of t-MN, but other factors such as social determinants of health also have to be considered.

In our analysis, we noted that patients with pre-existing bone metastasis were less likely to be diagnosed with t-MN and more frequently had BC infiltrating the bone marrow. This finding is to be expected since bone is the most common site of metastasis in BC patients and the subsequent carcinomatosis can cause myelosuppression and pancytopenia.[32,47]

Finally, we integrated all the clinical and laboratory par-

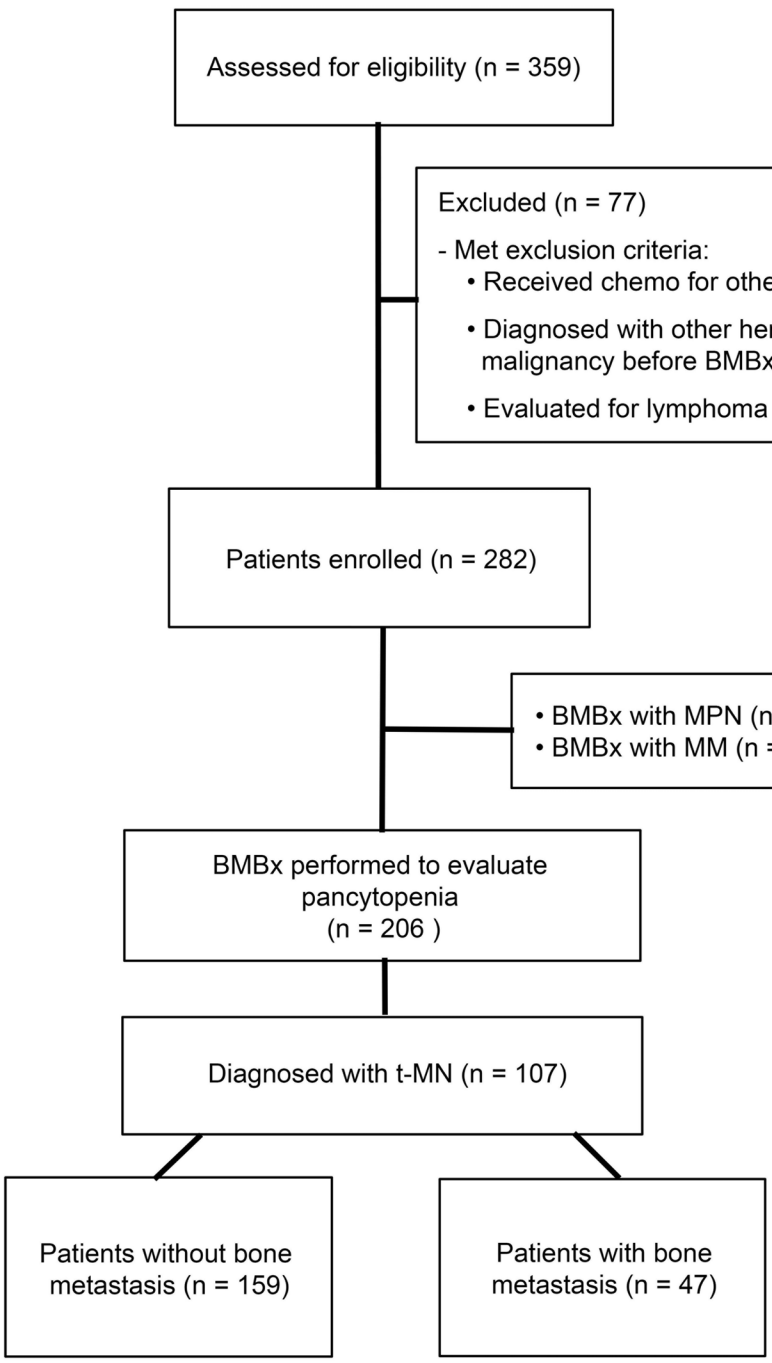

Figure 3. CONSORT diagram. Chemo: chemotherapy; BMBx: bone marrow biopsy; MPN: myeloproliferative neoplasms; MM: multiple myeloma; t-MN: therapy related myeloid neoplasms.

ameters to build a scoring system and determine a probability of having t-MN (Figure 3). This risk score is a practical tool that can be easily applied in the outpatient setting during scheduled follow-up and aid the clinician in predicting which patients are at higher risk of t-MN. Similar prediction models were created by Niu *at al.*[48] to assess the mortality risk in AML, and by Zhang *et al.*[49] to predict lymph node metastasis in gastric cancer patients. It needs to be specified that we reported *ad hoc* cutoff points that correspond to the minimum score of t-AML/MDS diagnosed patients. A larger prospective study is needed to reliably estimate a clinically relevant threshold.

A potential source of bias in this study includes the fact that our population only comprised patients referred to the hematology clinic, possibly because their breast oncologist sought further evaluation of the patient's cytopenias. The criteria for referral were subjective and based solely on the judgment of the oncologist. At the same time, the decision whether to perform a BMBx was also determined by the evaluation of the hematologist. This most likely accounts for the extremely high incidence of t-MN that is inconsistent with the incidence of 0.1% to 1.8% reported in prior works.[2,8,50] Moreover, our study involves only the population of one center and may not be applicable to other centers. However, our risk score based on specific cutoff points is likely to be more generalizable (Figure 3).

In conclusion, to date no valid method exists to predict t-MN in cytopenic patients previously treated for BC. Our findings suggest that clinical and laboratory parameters can be used to create a predictive model to identify patients at higher risk for t-MN and aid in the early diagnosis of t-MN. Since BC patients are commonly followed for many years after their initial diagnosis, we propose the implementation of a screening tool, using the specific cutoffs that we adopted, as a novel inexpensive approach to identify patients at risk of t-MN and to determine the timing of a BMBx. Along with the need for prospective validation of our variables as predictive markers, further clinical application of our model to a larger population is warranted. The future incorporation of evolving research on clonal hematopoiesis may further improve the accuracy of this model and assist in the early detection of t-MN.

Disclosures

No conflicts of interest to disclose.

Contributions

GP performed research, data collection, analysis and wrote the manuscript. CG performed research and wrote the manuscript. AD performed data analysis and wrote the manuscript. AK collected data. MER reviewed the manuscript. RP wrote the manuscript and supervised the study. EMS collected data, analyzed data, wrote the manuscript and supervised the study.

Data-sharing statement

For original data, please contact the corresponding author.

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