



Original article

Secondary myeloid neoplasms: bone marrow cytogenetic and histological features may be relevant to prognosis



Roberta Sandra da Silva Tanizawa^{a,*}, Maria Claudia Nogueira Zerbini^b,
Ricardo Rosenfeld^c, Cristina Aiko Kumeda^a, Raymundo Soares Azevedo^b,
Sheila Aparecida Coelho Siqueira^a, Elvira Deolinda Rodrigues Pereira Velloso^a

^a Universidade de São Paulo, Faculdade de Medicina, Hospital das Clínicas, São Paulo, SP, Brazil

^b Universidade de São Paulo, Faculdade de Medicina, São Paulo, SP, Brazil

^c Universidade Federal de São Paulo (UNIFESP), Hospital São Paulo, São Paulo, SP, Brazil

ARTICLE INFO

Article history:

Received 22 July 2015

Accepted 21 September 2016

Available online 22 December 2016

Keywords:

Myelodysplastic syndromes

Second malignancy

Second neoplasm

Secondary effect

Therapy-associated neoplasm ;

ABSTRACT

Background: Secondary myeloid neoplasms comprise a group of diseases arising after chemotherapy, radiation, immunosuppressive therapy or from aplastic anemia. Few studies have addressed prognostic factors in these neoplasms.

Method: Forty-two patients diagnosed from 1987 to 2008 with secondary myeloid neoplasms were retrospectively evaluated concerning clinical, biochemical, peripheral blood, bone marrow aspirate, biopsy, and immunohistochemistry and cytogenetic features at diagnosis as prognostic factors. The International Prognostic Scoring System was applied. Statistical analysis employed the Kaplan–Meier method, log-rank and Fisher's exact test.

Results: Twenty-three patients (54.8%) were male and the median age was 53.5 years (range: 4–88 years) at diagnosis of secondary myeloid neoplasms. Previous diseases included hematologic malignancies, solid tumors, aplastic anemia, autoimmune diseases and conditions requiring solid organ transplantations. One third of patients (33%) were submitted to chemotherapy alone, 2% to radiotherapy, 26% to both modalities and 28% to immunosuppressive agents. Five patients (11.9%) had undergone autologous hematopoietic stem cell transplantation. The median latency between the primary disease and secondary myeloid neoplasms was 85 months (range: 23–221 months). Eight patients were submitted to allogeneic hematopoietic stem cell transplantation to treat secondary myeloid neoplasms. Important changes in bone marrow were detected mainly by biopsy, immunohistochemistry and cytogenetics. The presence of clusters of CD117⁺ cells and p53⁺ cells were associated with low survival. p53 was associated to a higher risk according to the International Prognostic Scoring System. High prevalence of clonal abnormalities (84.3%) and thrombocytopenia (78.6%) were independent factors for poor survival.

* Corresponding author at: Cytogenetics Laboratory, Hematology Service, Hospital das Clínicas, Faculdade de Medicina, Universidade de São Paulo, Av Dr. Enéas de Carvalho Aguiar, 155, 1° andar, 05403-000 São Paulo, SP, Brazil.

E-mail address: robertatani@uol.com.br (R.S. Tanizawa).

<http://dx.doi.org/10.1016/j.bjhh.2016.09.015>

1516-8484/© 2016 Associação Brasileira de Hematologia, Hemoterapia e Terapia Celular. Published by Elsevier Editora Ltda. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Conclusion: This study demonstrated that cytogenetics, bone marrow biopsy and immunohistochemistry are very important prognostic tools in secondary myeloid neoplasms.

© 2016 Associação Brasileira de Hematologia, Hemoterapia e Terapia Celular. Published by Elsevier Editora Ltda. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Introduction

Secondary myeloid neoplasms (s-MN) comprise a group of diseases arising as late complications after chemotherapy and radiation and are associated with risk factors such as congenital disorders and acquired bone marrow (BM) failure.¹⁻⁴

The most studied of these s-MN are related to chemotherapy and radiotherapy. The rate of these diseases is increasing as the survival of patients with cancer improves. Patients submitted to autologous hematopoietic stem cell transplantation (HSCT) with intensive chemotherapy and total body irradiation (TBI), a type of complementary therapy, have demonstrated a potential of developing secondary myeloid disorders.⁵ The 2008 World Health Organization (WHO) classification adopted the term therapy-related myeloid neoplasms (t-MN) for cases of myeloid malignancies after chemotherapy/radiotherapy (CH/RT) that fulfill morphological criteria including myelodysplastic syndromes (MDS), acute myeloid leukemia (AML) and myeloproliferative neoplasms (MPN). Alkylating drugs/radiation and topoisomerase II inhibitor agents are implicated in these disorders. The characteristics of t-MN in the majority of cases include anemia with macrocytosis, dysplastic changes in neutrophils and basophilia. BM fibrosis, three lineage dysplasia, ring sideroblasts and abnormal karyotypes are seen in the majority of cases, with almost 50% of cases having less than 5% of BM blasts. The clinical course is typically progressive and resistant to conventional therapies.⁶

Long-surviving patients treated with immunosuppressive therapy, such as aplastic anemia (AA), autoimmune disease and recipients of solid organ grafts have an increased risk of developing s-MN.⁷⁻⁹ Furthermore, the concomitant use of immunosuppressive agents with hematopoietic growth factors has been associated with s-MDS.¹⁰ To date, little is known about s-MN after using immunosuppressive therapy. There are few data in the literature concerning morphologic and outcome studies. However, cytogenetic abnormalities are an important marker in this subgroup with anomalies usually involving chromosomes 6 and 8 and in particular monosomy 7.¹¹

Genetic diseases such as Fanconi anemia, dyskeratosis congenita, Diamond-Blackfan anemia, Shwachman-Diamond syndrome and some forms of severe congenital neutropenia have an increased propensity for myeloid neoplasms.¹² Furthermore, studies about host factors such as common polymorphisms in drug metabolizing enzymes and biological markers of drug- and radiation-induced genetic damage may be useful in identifying patients at risk of therapy-related complications and secondary malignancies.^{13,14}

Data concerning overall survival (OS) and prognosis of patients with s-MN remain poor. Allogeneic HSCT seems to be the only curative therapy for all subgroups included in s-MN.¹⁵

Cytogenetic status plays an important role in determining the outcome of these patients. Outcome for primary disease, platelet count, hemoglobin level, age, total protein level, C-reactive protein level and unfavorable karyotype have been described as prognostic factors in some studies.¹⁶⁻¹⁸ Despite the importance of BM studies in pathological processes of secondary disease, few investigations have been conducted concerning BM biopsies and immunohistochemistry. Orazi et al.¹⁹ analyzed 14 patients with previous diseases including Hodgkin's lymphoma, Non-Hodgkin lymphoma, breast cancer, plasma cell myeloma and skin carcinoma that evolved to therapy-related MDS. Data from BM biopsies, such as abnormal localization of immature precursors, marrow fibrosis, and overexpression of CD34⁺ cells have been associated with poor prognosis. In addition, p53 protein overexpression can be frequently observed, particularly in cases associated with severe ineffective hematopoiesis.²⁰

Objective

The aim of this study was to analyze clinical, biochemical, morphological (peripheral blood, BM aspirate and biopsy) and cytogenetic characteristics as prognostic factors in patients with s-MN diagnosed and treated at a single center.

Methods

Of 428 patients with MDS in the database of HC-FMUSP, a public hospital in São Paulo, Brazil, 42 patients (10%) with s-MN after the use of chemotherapy, radiotherapy (for hematological or solid tumors) or immunosuppressive therapy (for AA, solid transplantation and autoimmune diseases) from 1987 to 2008 were retrospectively studied. This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the institution. Medical charts were evaluated for clinical characteristics and laboratory data at diagnosis (Tables 1 and 2). BM aspirates were reviewed according to morphological criteria of the WHO 2008 criteria. BM biopsies were reviewed for cellularity, an estimated blast percentage, fibrosis and dyspoiesis. Immunohistochemistry was carried out for myeloperoxidase, Glycophorin A, CD61, FVIII, CD20, CD3, CD138, CD34, CD117 and p53 protein expressions. The latter was defined as 0 to 3+ and considered positive if any expression was detected (Table 2). Cytogenetics was performed at diagnosis using standard G-banding²¹ and karyotypes were classified as normal, abnormal, complex (≥ 3 abnormalities) or monosomy 7.²² The International Prognostic

Table 1 – Univariate analysis for prognostic factors on overall survival of patients with secondary myeloid neoplasms – clinical data.

	Number of cases	Median survival (months)	p-Value
Overall survival censoring allogeneic HSCT	42	5.7	
Overall survival not censoring allogeneic HSCT	42	6	
Gender (n = 42)			
Male	23	5.9	0.974
Female	19	5.1	
Age (n = 42)			
<50 years	18	8.7	0.135
≥50 years (range 4–88)	24	3.6	
Primary disease (n = 42)			
Hematologic malignancies	19	3.5	0.018
Solid tumors	6	3.6	0.833
Aplastic anemia	11	20.7	0.041
Autoimmune disorders and solid organ transplantations	6	12.2	0.651
Median latency (n = 41)			
<85 months (range 23–221)	20	3.6	0.264
≥85 months	21	9.2	
Allogeneic HSCT (n = 42)			
No	34	5.7	0.007
Yes	8	40	
Previous therapy (n = 39)			
CH	14	5.9	0.879
RT	1	3.6	0.599
CH + RT	11	2.5	0.749
Autologous HSCT	5	5.9	0.309
IST	12	7.1	0.764

CH: chemotherapy; HSCT: hematopoietic stem-cell transplantation; IST: immunosuppressive therapy; RT: radiotherapy.

Scoring System (IPSS) for primary MDS²³ was calculated. The IPSS has already been validated for therapy-induced MDS.²⁴

The data were analyzed using version 16.0 of the Statistical Package for the Social Sciences (SPSS) computer program. Statistical analysis for OS used the Kaplan–Meier product limit method, censoring the time of allogeneic HSCT, and log-rank test for comparing the OS between subgroups. The significant variables in univariate analysis (except for histological factors and biochemistry data because of the small number of samples) were submitted to the binary logistic regression test. Fisher's exact test was used for associations between categorical variables such as p53 and abnormal cytogenetics, blast cells and CD34⁺ and CD117⁺ cells.

Results

Twenty-three patients (54.8%) were male, with a median age of 53.5 years (range: 4–88 years) at the diagnosis of s-MN. Hematologic malignancies, solid tumors, AA, autoimmune diseases and recipients of solid organ transplantations were identified as previous conditions related to the disease. For previous therapy, 33% of patients had been submitted to chemotherapy alone, 2% to radiotherapy, 26% to both modalities and 28% to immunosuppressive agents. Five patients (11.9%) had undergone autologous HSCT. The patient characteristics are shown in Table 1 and further information about clinical data are available in a previous publication.²² The median latency between the primary disease and s-MN was 85 months (range: 23–221 months). Eight patients underwent allogeneic HSCT to treat s-MN (Table 1).

Peripheral blood analysis was performed in all cases and showed anemia, neutropenia and thrombocytopenia in more than 60% of the cases; more than 25% presented blast cells. Serum lactate dehydrogenase was increased in 41%, ferritin >1000 ng/mL in 50% and albumin <3.2 g/dL in 22.7% of the available cases (Table 2).

BM aspirates were available in 37 cases, with 38% of patients presenting blasts under 5%, 43% of patients with blasts between 5–19% and 19% of the cases with 20% of blasts or more. Two patients presented characteristics of MDS/myeloproliferative syndrome, one chronic myelomonocytic leukemia and one with thrombocytosis. Increases in eosinophil, basophil and plasma cell counts were detected in few cases, and >15% of ring sideroblasts in only 14.3% (Table 2).

Twenty-two BM biopsies analyzed with a median length of 1.4 cm (range: 0.6–3.7 cm) revealed global hypocellularity in 9.1%. Hypobulated megakaryocytic was the most relevant finding concerning dysplasia; increased fibrosis was detected in 62%, abnormal localization of immature precursors in 23.8%, lymphoid nodules in 40.9%, CD34⁺ and CD117⁺ cells were detected in more than 75% of the cases and elevated p53 protein expression in 33.3%. CD117, but not CD34, was often positive in the cytoplasm of megakaryocytes (Table 2).

Of 42 patients, 38 samples were available for cytogenetic analysis with six cases having no cell growth. Analysis was conclusive in 32 cases. Clonal abnormalities were observed in 84.4% of cases. A high prevalence of unbalanced abnormalities (96.3%) was observed and only one case presented with t(3;21)(q26;q22) as a single anomaly. The results of karyotype and fluorescence in situ hybridization (FISH) studies have already been published.²²

Table 2 – Univariate analysis for prognostic factors on overall survival of patients with secondary myeloid neoplasms – laboratory data.

	Number of cases	Median survival (months)	p-Value
Peripheral blood (PB)			
Hemoglobin (g/dL) – n = 42			
≥10.0	15	5.6	0.29
<10.0 (mean: 9, range 5.8–14.7)	27	5.9	
Neutrophils × 10 ⁹ /L – n = 42			
≥1.8	15	5.1	0.615
<1.8 (mean: 1.2, range 0.2–10.8)	27	5.9	
Platelets × 10 ⁹ /L – n = 42			
≥100	9	12.2	0.084
<100 (mean:43, range 7–368)	33	5.1	
Blasts in PB – n = 42			
Absent	31	5.9	0.295
Present	11	2.4	
Biochemical			
LDH (U/L) – n = 24			
≤480	14	9.2	0.002
>480 (mean: 414, range 271–4310)	10	1.7	
Albumin (g/dL) – n = 22			
≥3.2	17	5	0.624
<3.2 (mean: 3.6, range 2.1–4.5)	5	6.3	
Ferritin (ng/mL) – n = 16			
≤1000	8	6.3	0.037
>1000 (mean: 822, range 45–7842)	8	1.7	
Bone marrow aspirate			
Global cellularity – n = 37			
Hipocellularity	11	5.9	0.853
Normal	11	7.1	
Hipercellularity	15	3.6	
Cellularity MGK serie – n = 36			
Hipocellularity	19	5.6	0.79
Normal	9	5.1	
Hipercellularity	8	7.1	
Dysplasia MGK serie – n = 22			
No	7	12.9	0.233
Yes	15	5.1	
Pseudo-Pelger-Huët anomaly – n = 35			
No	25	6.3	0.586
Yes	10	3.6	
(%) BM Blasts – n = 37			
<5	14	5.1	0.825
≥5	23	5.6	
Eosinophils (%) – n = 37			
≤5	34	5.9	0.243
>5	3	3.5	
Basophils (%) – n = 37			
≤1	35	5.9	0.495
>1	2	3.5	
Plasma cells (%) – n = 37			
≤5	35	5.9	0.139
>5	2	2	
Bone marrow biopsy			
Global cellularity – n = 22			
Hipocellularity	2	12.7	0.948
Normal	7	5	
Hipercellularity	13	9.2	
Cellularity MGK serie – n = 22			
Hipocellularity	7	1.7	0.977
Normal	6	7.1	
Hipercellularity	9	9.2	
Dysplasia			
No	2	1.7	0.608
Yes	15	12.2	

Table 2 – (Continued)

	Number of cases	Median survival (months)	p-Value
Architectural changes			
No	4	19	0.464
MGK serie – n = 17			
Yes	13	9.2	
ALIP – n = 19			0.31
No	14	7.1	
Yes	5	20.7	
CD34 ⁺ cells – n = 22			0.181
≤1%	5	Not reached	
>1%	17	7.1	
CD34 ⁺ cells – n = 17			0.676
(1–10%)	12	8.7	
>10%	5	7.1	
CD34 ⁺ cluster – n = 22			0.755
No	9	9.2	
Yes	13	7.1	
CD117 ⁺ cells – n = 17			0.172
≤1%	3	19	
>1%	14	9.2	
CD117 ⁺ cells – n = 14			0.081
(1–10%)	9	12.3	
>10%	5	1.7	
CD117 ⁺ cluster – n = 17			0.029
No	12	12.7	
Yes	5	1.7	
p53 protein expression – n = 21			0.056
No	14	12.7	
Yes	7	3.5	
Lymphoid nodules – n = 22			0.81
No	13	12.2	
Yes	9	9.2	
Fibrosis – n = 21			0.608
<Grade 2	8	7.1	
≥Grade 2	13	9.2	
Cytogenetics			
Karyotype			
Normal vs abnormal – n = 32			0.030
Normal	5	Not reached	
Abnormal	27	5	
Complex vs others – n = 27			0.057
Complex	12	3.5	
Others	15	8.7	
Monosomy 7 vs complex – n = 22			0.123
Monosomy 7	10	5	
Complex	12	3.5	
IPSS risk – n = 31			0.038
Low + intermediate I	6	12.2	
Intermediate II + high	25	5	

ALIP: abnormal localization immature precursor; LDH: lactate-dehydrogenase; MGK: megakaryocytic.

In univariate analysis, hematologic malignancies, low platelet count, high serum lactate dehydrogenase and ferritin levels, detection of CD117 clusters, p53⁺, abnormal cytogenetics, Intermediate-II and high risk IPSS groups were found to be associated with poor survival. No parameter studied from BM aspirate had impact on survival (Table 2). Hematologic malignancies as a previous disease, low platelet count, abnormal karyotype (Table 2) and patients who had not undergone allogeneic HSCT were independent factors that predicted poorer survival in this sample. Figure 1 shows OS curves according to the most relevant prognostic findings in s-MN.

The binary logistic regression test, applied to 32 patients with complete clinical, peripheral blood, BM aspirate and cytogenetic parameters, showed that clonal abnormalities (p -value = 0.012) and platelet counts $<100 \times 10^9/L$ (p -value = 0.028) were independent factors for poor survival. Fisher's exact test showed no correlations between p53 and abnormal cytogenetics (p -value = 0.092), p53 and CD34⁺ cells (p -value = 0.554), p53 and CD117⁺ cells (p -value = 0.176); blast cells and CD34⁺ cells (p -value = 0.674), blast cell and CD34⁺ cell clusters (p -value = 0.583), blast cells and CD117⁺ cells (p -value = 0.604), and blast cell and CD117⁺ cell clusters (p -value = 0.126).

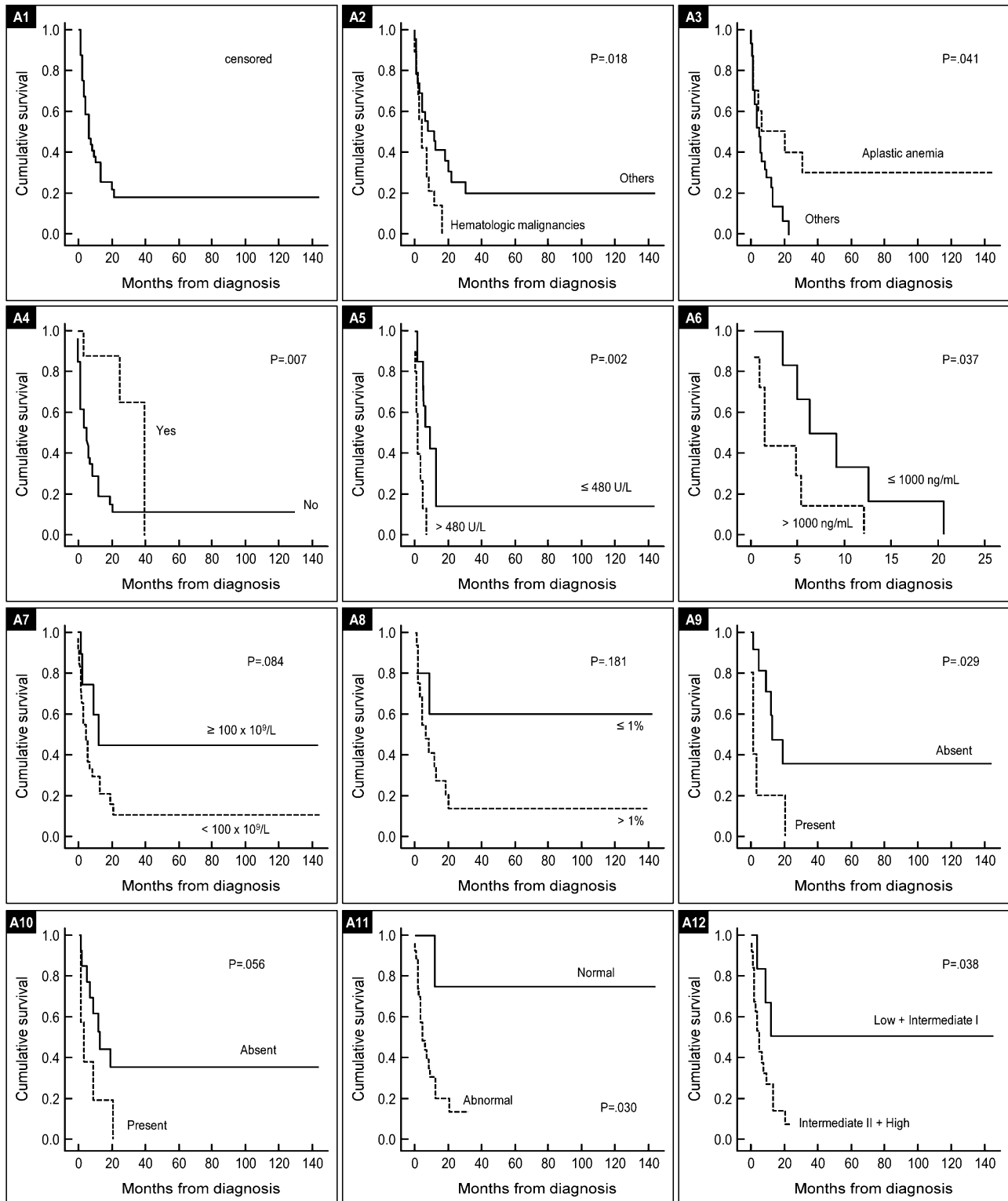


Figure 1 – Kaplan-Meier survival curves for secondary MN patients by significant poor prognostic factors.

(A1): Overall survival censoring allogeneic HSCT. (A2): Previous disease. (A3): Previous disease. (A4): Allogeneic HSCT for therapy. (A5): Serum LDH. (A6): Serum Ferritin. (A7): Platelets. (A8): CD34⁺ cells. (A9): CD117⁺ cluster. (A10): p53 protein expression. (A11): Karyotype. (A12): IPSS-Risk.

Discussion

Nowadays the number of s-MN has become increasingly important. This increase is mainly related to an expanded use of high-dose chemotherapy/radiotherapy. The risk of developing s-MN after immunosuppressive therapy seems important as well since several cases have been reported so far in the literature.⁷⁻¹¹ This is a retrospective study with a small and heterogeneous sample; it reflects consecutive cases of s-MN over a period of 21 years diagnosed and treated in a single center.

This study included patients submitted to different previous therapies (CH/RT and immunosuppressive therapy); the findings of this study are consistent with the clinical picture seen in patients with t-MN. The frequency of s-MN in this study was 10% of cases in the MDS database, with a median age of 53 years. The most common previous disease was plasma cell myeloma treated with melphalan (21.4%), and there were three patients with follicular lymphoma treated with fludarabine associated with cyclophosphamide, reinforcing the theory that this association increases the risk of developing t-MN as suggested in a previous study.²⁵ Breast cancer was the most frequent solid tumor (50% – 4/8) consistent with published data that has reported an increased rate of s-MN in this group of survivors probably as a consequence of an effective therapy.^{26,27}

The median OS for all patients was six months, basically the same observed in other series of patients with t-MN that reported four and eight months.^{15,28} There was a significant difference between patients who were submitted to allogeneic HSCT (40 months) and those who were not (5.7 months) (p -value = 0.007). This is in agreement with published data and showed the crucial importance of this modality of therapy.

The findings of this study suggest that BM aspirates provide limited results, probably due to aspirate dilution, and did not contribute to the prognosis of these patients. The percentage of blasts in BM aspirate was not significantly associated with survival. This result might be explained by the poor quality of the sample material and the percentage of blasts may be underestimated, since the value was not representative in one third of cases.

Individuals with previous AA seem to be a different subset of patients, showing peculiar characteristics as in this sample a longer median period of latency and higher prevalence of monosomy 7 were seen. An interesting discussion point is whether these patients had this cytogenetic abnormality before treatment or not.

The importance of integrated data analysis was demonstrated in this study with five patients, whose evaluations of BM aspirates were not available. Of these, two had complex karyotypes. The remaining three patients presented remarkable dysplasia in at least granulocyte and megakaryocyte lineages, fibrosis (Grade 2-3) and CD34⁺ or CD117⁺ cells (1-10%) revealed in BM biopsies and immunohistochemistry. The importance of BM immunohistochemistry should be stressed in respect to the p53 protein expression (Figure 2) and CD117/CD34 blast evaluations to identify the presence of aggregates of positive cells (Figures 3 and 4).

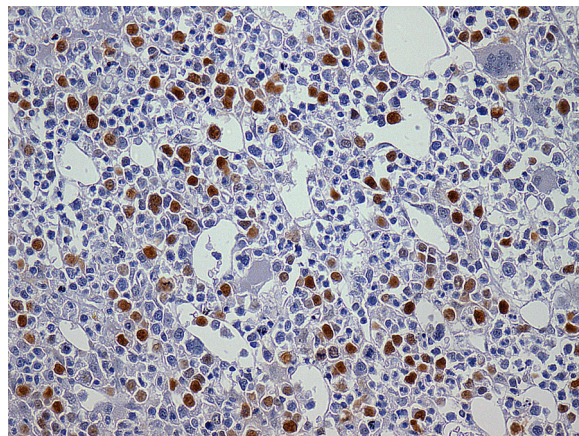


Figure 2 – Bone marrow biopsy of patient with secondary myeloid neoplasm showing p53+ (immunohistochemical stains - 400×), previous disease: breast tumor.

BM biopsies showed hypobulated megakaryocytes as the most relevant finding concerning dysplasia (Figure 5). CD117⁺ clusters and p53 expression had an impact on the survival of patients with median survival of only 1.7 and 3.5 months, respectively. OS curves for patients with CD34⁺ versus CD34⁻ cells, did not reach statistical significance, probably because of the small number of patients. The correlation between p53⁺ and abnormal cytogenetics did not reach statistical significance probably due to small number of cases. Alterations of p53⁺ have been found in association with aggressive disease,¹⁸ and larger series of cases are necessary to confirm our observation of CD117 clusters as a prognostic marker for this condition.

The importance of cytogenetic analysis in diagnosis and prognosis of myeloid neoplasms is well documented. The results of this study are consistent with the literature^{29,30} and showed that normal cytogenetics was associated to better survival (p -value=0.03) and abnormal karyotype was an independent risk factor for poor survival (p -value=0.012).

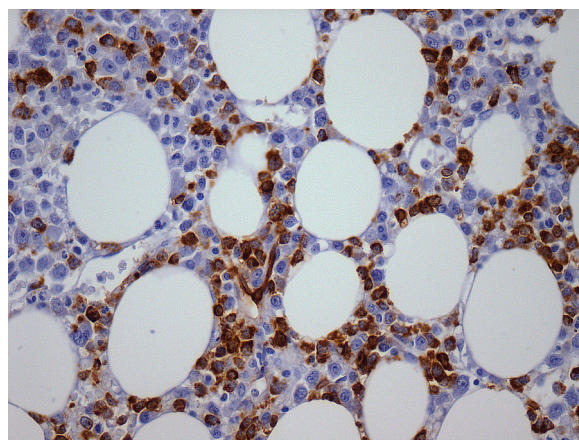


Figure 3 – Bone marrow biopsy of patient with secondary myeloid neoplasm showing cluster CD34+ cells (immunohistochemical stains - 400×), previous disease: follicular lymphoma.

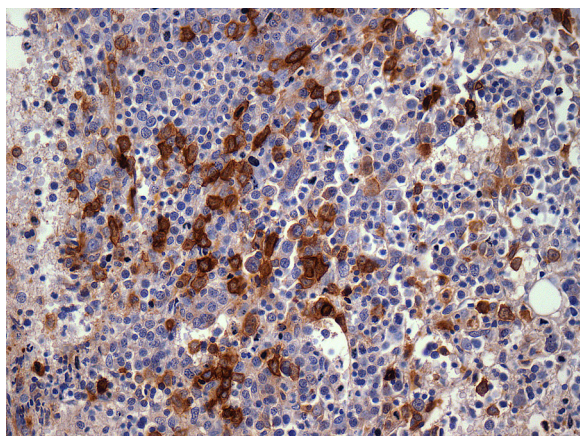


Figure 4 – Bone marrow biopsy of patient with secondary myeloid neoplasm showing cluster CD117+ cells (immunohistochemical stains - 400x), previous disease: severe aplastic anemia.

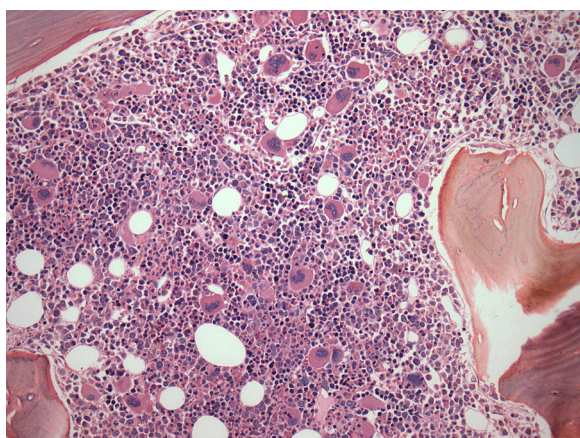


Figure 5 – Bone marrow biopsy of patient with secondary myeloid neoplasm showing dysplasia and architectural changes in MGK serie (Hematoxylin and eosin stain - 200x), previous renal transplant.

In addition, independent factors in multivariate analysis for poor survival included thrombocytopenia, abnormal karyotype and absence of allogeneic HSCT as therapy. Hypoproteinemia was not a poor prognostic factor in this investigation, as shown in a previous Japanese study.¹⁸

Conclusion

In summary, factors associated in univariate analysis with poor survival included previous oncohematological diseases, thrombocytopenia, elevated lactate dehydrogenase and ferritin levels, CD117⁺ clusters, p53⁺, abnormal karyotype, IPSS risk (intermediate II and high subgroups) and the absence of allogeneic HSCT as therapy. Despite the fact that IPSS was not designed for secondary MDS, it seems to be useful in this situation, mainly in respect to thrombocytopenia and abnormal karyotypes, parameters with high prevalence in MDS (79% and 84% of patients respectively in this study).

Prevention of this late complication of primary treatment programs must be continuously re-evaluated. Further studies with a larger number of cases of s-MN should be conducted to improve the understanding of the pathophysiologic mechanisms of the disease and the determination of biomarkers, in order to diagnose and treat this aggressive disease.

Conflict of interest

The authors declare no conflicts of interest.

REFERENCES

1. Fianchi L, Pagano L, Piciocchi A, Candoni A, Gaidano G, Breccia M, et al. Characteristics and outcome of therapy-related myeloid neoplasms: report from the Italian network on secondary leukemias. *Am J Hematol*. 2015;90(5):E80–5.
2. Luna-Fineman S, Shannon KM, Lange BJ. Childhood monosomy 7: epidemiology, biology, and mechanistic implications. *Blood*. 1995;85(8):1985–99.
3. Imashuku S, Hibi S, Bessho F, Tsuchida M, Nakarata T, Myiazaki S, et al. Detection of myelodysplastic syndrome/acute myeloid leukemia evolving from aplastic anemia in children treated with recombinant human G-CSF. *Haematologica*. 2003;88(11):136–41.
4. Churpek JE, Larson RA. The evolving challenge of therapy-related myeloid neoplasms. *Best Pract Res Clin Hematol*. 2013;26(4):309–17.
5. Lenz G, Dreyling M, Schiegnitz E, Haferlach T, Hasford J, Unterhalt M, et al. Moderate increase of secondary hematologic malignancies after myeloablative radiochemotherapy and autologous stem-cell transplantation in patients with indolent lymphoma: results of a prospective randomized trial of the German Low Grade Lymphoma Study group. *J Clin Oncol*. 2004;22(24):4926–33.
6. Vardiman JW, Arber DA, Brunning RD, Larson RA, Matutes E, Baumann I, et al. Therapy-related myeloid neoplasms. In: Swerdlow SH, Campo E, Harris NL, et al, editors. *WHO classification of tumours of haematopoietic and lymphoid tissues*. Lyon: IARC; 2008. p. 127–9.
7. McCarthy CJ, Sheldon S, Ross CW, McCune WJ. Cytogenetics abnormalities and therapy related myelodysplastic syndromes in rheumatic disease. *Arthritis Rheum*. 1998;41(8):1493–6.
8. Huebner G, Karthaus M, Pethig K, Freund M, Ganser A. Myelodysplastic syndrome and acute myelogenous leukemia secondary to heart transplantation. *Transplantation*. 2000;70(4):688–90.
9. Abu-Shakra M, Buskila D, Ehrenfeld M, Conrad K, Shoenfeld Y. Cancer and autoimmunity: immune and rheumatic features in patients with malignancies. *Ann Rheum Dis*. 2001;60(5):433–41.
10. Gurion R, Gaftor-Gvilli A, Paul M, Vidal L, Ben-Bassat I, Yeshurun M, et al. Hematopoietic growth factors in aplastic anemia patients treated with immunosuppressive therapy-systematic review and meta-analysis. *Hematologica*. 2009;94(5):712–9.
11. Maciejewski JP, Selleri C. Evolution of clonal cytogenetic abnormalities in aplastic anemia. *Leukemia Lymphoma*. 2004;45(3):433–40.
12. Parikh S, Bessler M. Recent insights into inherited bone marrow failure syndromes. *Curr Opin Pediatr*. 2012;24(1):23–32.
13. Voso MT, Fabiani E, D'alo F, Guidi A, Di Ruscio A, Sica S, et al. Increased risk of acute myeloid leukaemia due to

- polymorphisms in detoxification and DNA repair enzymes. *Ann Oncol.* 2007;18(9):1523-8.
14. Bozina N, Brandamante V, Lovric M. Genetic polymorphism of metabolic enzymes P450 (CYP) as a susceptibility factor for drug response, toxicity and cancer risk. *Ahr Hig Rada Toksikol.* 2009;60(2):217-42.
 15. Smith SM, Le Beau MM, Huo D, Karrison T, Sobecks RM, Anastasi J, et al. Clinical cytogenetic associations in 306 patients with therapy-related myelodysplasia and myeloid leukemia: the University of Chicago series. *Blood.* 2003;102(1):43-52.
 16. Pedersen-Bjergaard J, Philip P, Larsen SO, Jensen G, Byrting K. Chromosome aberrations and prognostic factors in therapy-related myelodysplasia and acute nonlymphocytic leukemia. *Blood.* 1990;76(6):1083-91.
 17. Kantarjian HM, Keating MJ, Walters RS, Smith TL, Cork A, McCredie KB, et al. Therapy-related leukemia and myelodysplastic syndrome: clinical, cytogenetic, and prognostic features. *J Clin Oncol.* 1986;4(12):1748-57.
 18. Takeyama K, Seto M, Uike N, Hamajima N, Ino T, Mikuni C, et al. Therapy-related leukemia and myelodysplastic syndrome: a large-scale Japanese study of clinical and cytogenetics features as well as prognostic factors. *Int J Hematol.* 2000;71(2):144-52.
 19. Orazi A, Cattoretti G, Soligo D, Luksch R, Lambertenghi-Delilier G. Therapy-related myelodysplastic syndromes: FAB classification, bone marrow histology, and immunohistology in the prognostic assessment. *Leukemia.* 1993;7(6):838-47.
 20. Orazi A. Histopatologia in the diagnosis and classification of acute myeloid leukemia, myelodysplastic syndromes, and myelodysplastic/myeloproliferative diseases. *Pathobiology.* 2007;74(2):97-114.
 21. Yunis JJ, Sawyer JR, Ball DW. The characterization of high-resolution G-banded chromosomes of man. *Chromosoma.* 1978;67(4):293-307.
 22. Tanizawa RS, Kumeda CA, Azevedo Neto RS, Leal AM, Ferreira PB, Velloso EDRP. Karyotypic and fluorescent in-situ hybridization study of the centromere of chromosome 7 in secondary myeloid neoplasms. *Rev Bras Hematol Hemoter.* 2011;33(6):425-31.
 23. Greenberg P, Cox C, LeBeau MM, Fenaux P, Morel P, Sanz G, et al. International scoring system for evaluating prognosis in myelodysplastic syndromes. *Blood.* 1997;89(6):2079-88.
 24. Quintás-Cardama A, Daver N, Kim H, Dinardo C, Jabbour E, Kadia T, et al. A prognostic model of therapy-related myelodysplastic syndrome for predicting survival and transformation to acute myeloid leukemia. *Clin Lymphoma Myeloma Leuk.* 2014;14(5):401-10.
 25. Tam CS, Seymour JF, Prince HM, Kenealy M, Wolf M, Januszewicz EH, et al. Treatment-related myelodysplasia following fludarabine combination chemotherapy. *Hematologica.* 2006;91(11):1546-50.
 26. Park MJ, Park YH, Ahn HJ, Choi W, Paik KH, Kim JM, et al. Secondary hematological malignancies after breast cancer chemotherapy. *Leuk Lymphoma.* 2005;46(8):1183-8.
 27. Le Deley MC, Suzan F, Cutuli B, Delalogue S, Shansaldin A, Linassier C, et al. Anthracyclines, mitoxantrone, radiotherapy and granulocyte colony-stimulating factor: Risk factors for leukemia and myelodysplasia syndrome after breast cancer. *J Clin Oncol.* 2007;25(3):292-300.
 28. Josting A, Wiedenmann S, Franklin J, May M, Sieber M, Wolf J, et al. Secondary myeloid leukemia and myelodysplastic syndromes in patients treated for Hodgkin's disease: a report from the German Hodgkin's Lymphoma Study Group. *J Clin Oncol.* 2003;21(18):3440-6.
 29. Pedersen-Bjergaard J, Andersen MT, Andersen MK. Genetic pathways in the pathogenesis of therapy-related myelodysplasia and acute myeloid leukemia. *Hematology Am Soc Hematol Educ Program.* 2007:392-7.
 30. Andersen MK, Johansson B, Larsen SO, Pedersen-Bjergaard J. Chromosomal abnormalities in secondary MDS and AML. Relationship to drugs and radiation with specific emphasis on the balanced rearrangements. *Haematologica.* 1998;83(6):483-8.