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ORIGINAL ARTICLE

Risk stratification using a new prognostic score for patients with secondary acute myeloid leukemia: results of the prospective AML96 trial

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Patients with secondary acute myeloid leukemia (sAML) are generally thought to have a poor prognosis. As there are no prognostic risk stratification models for patients with sAML available, the aim of this study was to obtain a scoring system. Prognostic factors influencing overall survival (OS) and event-free survival (EFS) were analyzed in 305 sAML patients treated in the prospective AML96 trial. The obtained prognostic scoring system was then validated in an independent patient cohort included in the AML2003 and AML60+ trials. In addition to the known risk factors for AML, age and karyotype, we identified the absolute platelet count and the *Nucleophosmin 1* mutational status at diagnosis as prognostic factors of sAML patients. A pronounced distribution of sAML patients into three score groups was achieved showing a 2-year OS/EFS of 52/44% for patients in the low-risk group, 21/12% in the intermediate-risk group and 7/3% in the high-risk group (both $P<0.001$). Validation of this scoring system in a second independent set of sAML patients revealed similar significantly different survival results. In conclusion, for the first time, a prognostic scoring system is provided for sAML patients, allowing differential treatment strategies in the future.
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Introduction

Secondary acute myeloid leukemia (sAML) following a myelodysplastic syndrome (MDS) or deriving as therapy-related AML (tAML) after cytotoxic therapy is generally thought to have a poor prognosis. Following MDS, mdsAML account for 60–70% of all sAML, with tAML being responsible for the majority of the remaining cases.[1] About 30% of the patients with primary MDS develop mdsAML with progression rates to overt leukemia varying within the different MDS subgroups.[2] MDS-derived AML occurs mainly in elderly patients and has a poor prognosis with a lower rate of achieving complete remission (CR) than other AML subtypes.[3]

Following chemo- and/or radiotherapy, tAML represents ~10–20% of all the AML patients.[4] The risk associated with alkylating agents or radiation therapy increases with age, whereas the risk of developing tAML remains similar across all age groups after treatment with topoisomerase–II inhibitors.[5] Mutations induced by cytotoxic therapy, genetic predispositions that affect drug metabolism and DNA repair are implicated in the etiology of tAML.[6] The poor prognosis is based on cytogenetic abnormalities and the comorbidities of the underlying malignancy or acquired during previous cytotoxic therapy. Only 10–15% of the patients with *de novo* MDS or AML harbor the unbalanced aberrations 5q-/-5 or 7q-/-7, whereas in patients with tMDS or tAML, the incidence rises to 50–60%. In contrast, recurrent balanced translocations or inversions are observed in 10–20% of the patients with *de novo* as well as tAML. Normal karyotypes are present in an estimated 50% of the patients with *de novo* MDS and AML as compared with only 10–15% of the cases with tMDS and tAML.

Therapy-derived AML and mdsAML patients are often elderly and are diagnosed in a decreased performance status. Therefore, individual prognostic categorizations and treatment options are needed. In AML, general prognostic factors, such as age and cytogenetics, have been well established; however, for patients with sAML, there are no prognostic risk stratification models available.[7] Owing to progress in elucidating the impact of cytogenetic and molecular markers and therefore combining biological and clinical data for prognosis and treatment outcomes, the aim of this retrospective analysis was to provide a prognostic scoring system by including clinical and laboratory data from patients being treated in the prospective AML96 trial of the Study Alliance Leukemia (SAL) study group. Furthermore, verification of the applicability of this prognostic score by validation in an independent test set of patients was performed.

Patients and methods

The study population was comprised of 305 patients, aged 20–79 years, with mdsAML ($n=233$) and tAML ($n=72$), who were treated within the prospective AML96 trial of the SAL (formerly German Study Initiative Leukemia (DSIL)) from 1996 to 2003. In this trial, a total of 1916 patients with *de novo* or secondary AML were included. All sAML patients out of the AML96 trial were included into the presented study population here, with the exception for cytogenetically low-risk sAML patients, as they accounted for only $n=4$ and therefore no statistical conclusion for this cohort was expected.

MDS-derived AML was defined by at least one documented bone marrow examination, revealing MDS at least 3 months before the diagnosis of mdsAML. tAML was defined as an AML developing after cytotoxic chemotherapy and/or radiation

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therapy administered for a previous neoplastic or non-neoplastic disorder. Cytogenetic risk was defined as: (a) high risk: -5/del (5q); -7/del (7q); monosomies of other chromosomes (with exception for the loss of chromosomes X or Y); inv(3q); abn12p; abn11q; +11; +13; +21; +22; t(6;9); t(9;22); t(3; 3); complex aberrant karyotype (⩾three independent abnormalities); (b) standard risk: all patients not harboring high- or low-risk aberrations or (c) low risk: t(8;21) or inv(16) as previously described.[8] Cytogenetically low-risk sAML patients were excluded, as they accounted for only $n=4$. Patients aged ⩽60 years received intravenous double-induction chemotherapy containing cytosine arabinoside (100 mg/m^2, days 1–8), mitoxantrone (10 mg/m^2, days 4–8) and etoposide (100 mg/m^2, days 4–8) (=MAV) in the first induction and cytosine arabinoside (2 × 1000 mg/m^2, days 1–5) and m-AMSA (100 mg/m^2, days 1–5) (=MAMAC) during the second induction therapy. Patients with intermediate cytogenetic risk and an HLA-identical sibling donor were referred for allogeneic hematopoietic stem cell transplantation (HSCT). Patients without a sibling donor and patients with low cytogenetic risk were randomized to receive consolidation therapy consisting of either cytosine arabinoside (2 × 1000 mg/m^2, days 1–6) and mitoxantrone (10 mg/m^2, days 4–6) (=I-MAC) or cytosine arabinoside (2 × 3000 mg/m^2, days 1–6) and mitoxantrone (10 mg/m^2, days 4–6) (=H-MAC). Patients harboring high-risk cytogenetics were referred for related or unrelated allogeneic HSCT. In the case of unavailability of a donor, treatment was performed by using either I-MAC or H-MAC and subsequent autologous HSCT. Patients aged >60 years received induction chemotherapy containing daunorubicin (45 mg/m^2, days 3–5) and cytosine arabinoside (100 mg/m^2, days 1–7) (=DA45). In case of good or intermediate response to the first induction, a second induction (DA II) was performed. Patients in CR and good performance status received a consolidation therapy consisting of MAMAC.

Bone marrow and peripheral blood samples were obtained at diagnosis. Genomic DNA or RNA was extracted from mononuclear cells as published.[9,10] Polymerase chain reaction for *Nucleophosmin 1 (NPM1)* exon 12 and *FLT3-ITD* mutational analyses were performed as previously published.[10,11]

For validation of the obtained prognostic scoring system for sAML patients from the AML96 trial, the score was applied to an independent sample (test set) of sAML patients from the prospective AML2003− and AML60+ trials of the SAL. Eligibility for inclusion in the AML2003 trial was only for patients of 60 years and younger. Patients included in the AML2003 trial received double-induction chemotherapy containing daunorubicin (60 mg/m^2, days 3–5), cytosine arabinoside (100 mg/m^2, days 1–7) (=DA60) and subsequently risk-adapted postremission therapy with options for chemoconsolidation, autologous HSCT and allogeneic HSCT. Eligibility for inclusion in the AML60+ trial was only for patients older than 60 years. Patients included in the AML60+ trial received, when randomized into arm (A), induction chemotherapy with DA45 and, in case of good response, followed by a second induction of DA45 and chemoconsolidation containing MAMAC. When randomized into arm (B), induction chemotherapy with mitoxantrone (10 mg/m^2, days 1–3) and cytosine arabinoside (1000 mg/m^2, days 1, 3, 5, 7) (=I-MA) was performed. In case of a partial remission, a second induction of I-MA was performed, whereas consolidation therapy consisted of MAMAC.

All studies were approved by the ethical board of the Technical University Dresden. All the three protocols are in agreement with the Helsinki declaration and registered with the NCT numbers 00180115, 00180102 and 00180167. Written informed consent was obtained from all registered patients.

Statistical analysis

Treatment response and treatment outcome were defined according to the recommended consensus criteria.[12] Remission status was evaluated after two induction cycles. Overall survival (OS) end points, measured from the date of entry onto trial, were death and alive at last follow-up (censored). Event-free survival (EFS) endpoints, measured from the date of entry onto trial, were treatment failure, disease relapse or patient death from any cause at last follow-up (censored).[12] Consolidation therapy was classified into allogeneic HSCT, autologous HSCT and high-dose cytosine arabinoside-based chemotherapy. Clinical variables of statistical significance between mdsAML and tAML were compared using the χ^2-test for dichotomized variables and the *U*-test by Mann–Whitney for continuous variables. Logistic regression analyses were used to identify prognostic variables for CR rates. The method of Kaplan–Meier was used to estimate OS and EFS. Confidence interval (CI) estimation for the survival curves was based on the cumulative hazard function using the Greenwood's formula for the s.e. estimation. Survival distributions were compared using the log-rank test. Prognostic factors for survival were analyzed in a multiple, multivariate Cox regression model for OS stratified by treatment groups (chemoconsolidation versus allogeneic HSCT). Model selection was performed by backward selection applying the likelihood ratio test. In order to provide quantitative information on the relevance of results, 95% CI of odds ratios (ORs) and hazard ratios (HRs) were calculated. The prognostic score was derived from the multivariate Cox model for OS and EFS of all patients with sAML from the AML96 trial. Missing values for platelets at diagnosis ($n=2$) were accounted to the group of >50 Gpt/l, missing *NPM1* data ($n=92$) were accounted as being negative, whereas patients with missing cytogenetic subgroup data ($n=37$) were accounted to the standard-risk group as a conservative approach, as low-risk patients were excluded from the analysis. The prognostic score was then validated in a multivariate Cox model for OS of all the sAML patients with complete *NPM1* mutational status data ($n=213$) from the AML96 trial. Age as an internationally accepted risk factor was dichotomized at ⩽60 versus >60 years. For the platelet count, the clinically relevant cutpoint of ⩽50 versus >50 Gpt/l was defined, as >50 Gpt/l resembles the cutoff announced in the recommended clinical practice guidelines for invasive procedures in patients with cancer and hematologic diseases.[13,14] Furthermore a median platelet count of 49 Gpt/l has been proposed recently for the first time as a prognostic cutpoint for AML patients.[15] To assess the prognostic validity, a leave-one-out cross-validation was performed to estimate the cross-validation likelihood for the chosen factors. All factors were judged to be of significant prognostic value. All statistical analyses were performed using SPSS Version 16.0.1 (SPSS Inc., Chicago, IL, USA).

Results

Patient characteristics

The clinical characteristics and laboratory data at diagnosis of the 233 patients with mdsAML and 72 patients with tAML from the AML96 trial are depicted in Table 1. The median follow-up for patients alive was 5.3 years (range 0.01–9.65). For patients with tAML, the preceding malignancies are shown in Supplementary Table 1. The three patients receiving cytotoxic therapy because of non-neoplastic disease presented with autoimmune thyroiditis, polymyositis and systemic lupus erythematosus. The majority of patients with tAML had

Table 1 Patient characteristics of study set (AML96 trial) and validation set (AML2003 and AML60+ trials)

	AML96 sAML, n = *305*		*AML2003/60+ sAML*, n = *196*	
	mdsAML, n = *233*	*tAML*, n = *72*	*mdsAML*, n = *146*	*tAML*, n = *50*
Median age at diagnosis (range)	64 (24–79)	57 (20–78)	61.5 (30–82)	50.5 (18–72)
Gender, no. (%)				
Female	111 (48)	42 (58)	66 (45)	33 (66)
Male	122 (52)	30 (42)	80 (55)	17 (34)
Median bone marrow blasts at diagnosis, % (range)	50 (20.0–95.0)	56.5 (20.0–98.5)	39.5 (3–95)	52 (15.5–94.5)
Median WBC count at diagnosis, Gpt/l (range)	5.3 (0.2–296.7)	4.6 (0.5–286)	4.38 (0.4–148.5)	9.8 (0.19–242)
Median platelet count at diagnosis, Gpt/l (range)	41 (2–1043)	50 (4–433)	52 (2–1308)	47 (7–620)
Median hemoglobin at diagnosis, g per 100 ml (range)	9.2 (3.6–15.4)	8.7 (3.9–13.4)	9.3 (5.4–16.75)	9.5 (5.7–12.9)
Median CD34+ bone marrow blasts at diagnosis, % (range)	37 (0–96)	17 (0–97)	25.6 (0–97.3)	7.75 (0–61)
Median POX positive blasts at diagnosis, % (range)	20.5 (0–100)	25.0 (0–100)	29 (0–100)	11 (0–100)
Median serum LDH at diagnosis, U/l (range)	315 (97–4480)	259 (10–2178)	294 (113–3058)	552 (187–10000)
FLT3 mutational status, no. (%)				
FLT3-ITD positive	26 (15)	6 (12)	13 (12)	3 (7)
FLT3-ITD negative	148	45	98	39
NPM1 mutational status, no. (%)				
NPM1 mut	27 (17)	3 (6)	16 (15)	5 (12)
NPM1 wt	136	47	90	36
NPM1/FLT3 grouped				
NPM mut/FLT3-ITD negative	14 (9)	1 (2)	13 (12)	4 (10)
NPM mut/FLT3-ITD positive	13 (8)	2 (4)	3 (3)	1 (2)
NPM wt/FLT3-ITD positive	12 (7)	4 (8)	9 (8)	2 (5)
NPM wt/FLT3-ITD negative	120 (75)	41 (85)	81 (76)	33 (82)
ECOG status at diagnosis, no. (%)				
0–2	163 (93)	44 (90)	110 (98)	42 (93)
3–4	13 (7)	5 (10)	2 (2)	3 (7)
FAB subtypes at diagnosis no. (%)				
M0	14 (6)	5 (7)	6 (5)	2 (4)
M1	42 (19)	14 (20)	18 (15)	6 (13)
M2	87 (39)	19 (27)	54 (44)	9 (19)
M4	33 (15)	10 (14)	9 (7)	7 (15)
M4eo	2 (1)			
M5a	15 (7)	12 (17)	5 (4)	13 (28)
M5b	4 (2)	3 (4)	1 (0)	3 (6)
M6	12 (5)	4 (6)	6 (5)	2 (4)
M7	2 (1)	1 (1)	3 (2)	
RAEB-T	11 (5)	3 (4)	22 (18)	5 (11)
Cytogenetic subgroups, no. (%)				
High-risk AML	64 (27)	26 (36)	44 (30)	16 (32)
Standard-risk AML	169 (73)	46 (64)	102 (70)	34 (68)
CR after second induction, no. (%)	59 (25)	29 (40)	38 (26)	12 (24)

Abbreviations: AML, acute myeloid leukemia; CR, complete remission; ECOG, Eastern Cooperative Oncology Group; FAB, French–American–British association; FLT3, fms-like tyrosine kinase 3; ITD, internal tandem duplication; LDH, lactate dehydrogenase; MDS, myelodysplastic syndrome; mdsAML, AML with preceding MDS; NPM1, nucleophosmin 1; POX, peroxidase; sAML, secondary AML; tAML, therapy-related AML; WBC, white blood cells.

alkylating agents and/or topoisomerase II inhibitors and/or radiation therapy as cytotoxic treatment due to preceding disease. These data are depicted in Supplementary Table 2. Cytogenetic high-risk aberrations were found in 64 (27%) patients with mdsAML and 26 (36%) patients with tAML (Table 1). Treatment courses, patient numbers and outcomes are displayed in modified consort flow diagrams in Figure 1 and 2 for the AML96−, AML2003− and AML60+ trials, respectively.

Patients with mdsAML were older compared with patients with tAML (Table 1), with a median of 64 versus 57 years, $P<0.001$, respectively. The median $CD34^+$ blast count in the bone marrow was higher in patients with mdsAML as compared with their tAML counterparts (37 versus 17%, $P=0.003$). The median platelet count was similar in both groups at diagnosis (41 Gpt/l in patients with mdsAML and 50 Gpt/l in patients with tAML).

Treatment outcome

CR rate after double induction therapy for all patients was 33% ($n=88$).

The following variables at diagnosis and after first induction therapy were evaluated for their potential influence on CR and survival: age, disease status (mdsAML versus tAML), WBC count, serum lactate dehydrogenase, platelet count, $CD34^+$ bone marrow blasts at diagnosis, cytogenetic subgroups (standard risk

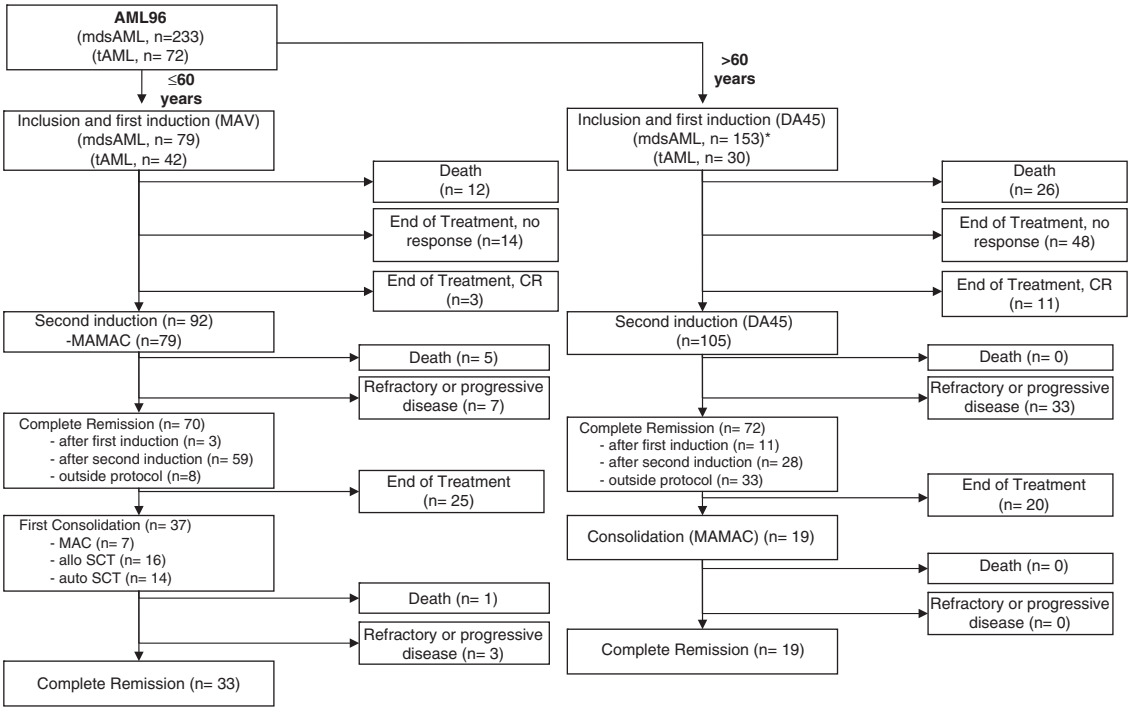

Figure 1 Modified consort flow diagram showing patient numbers, treatments and outcomes of the AML96 trial.

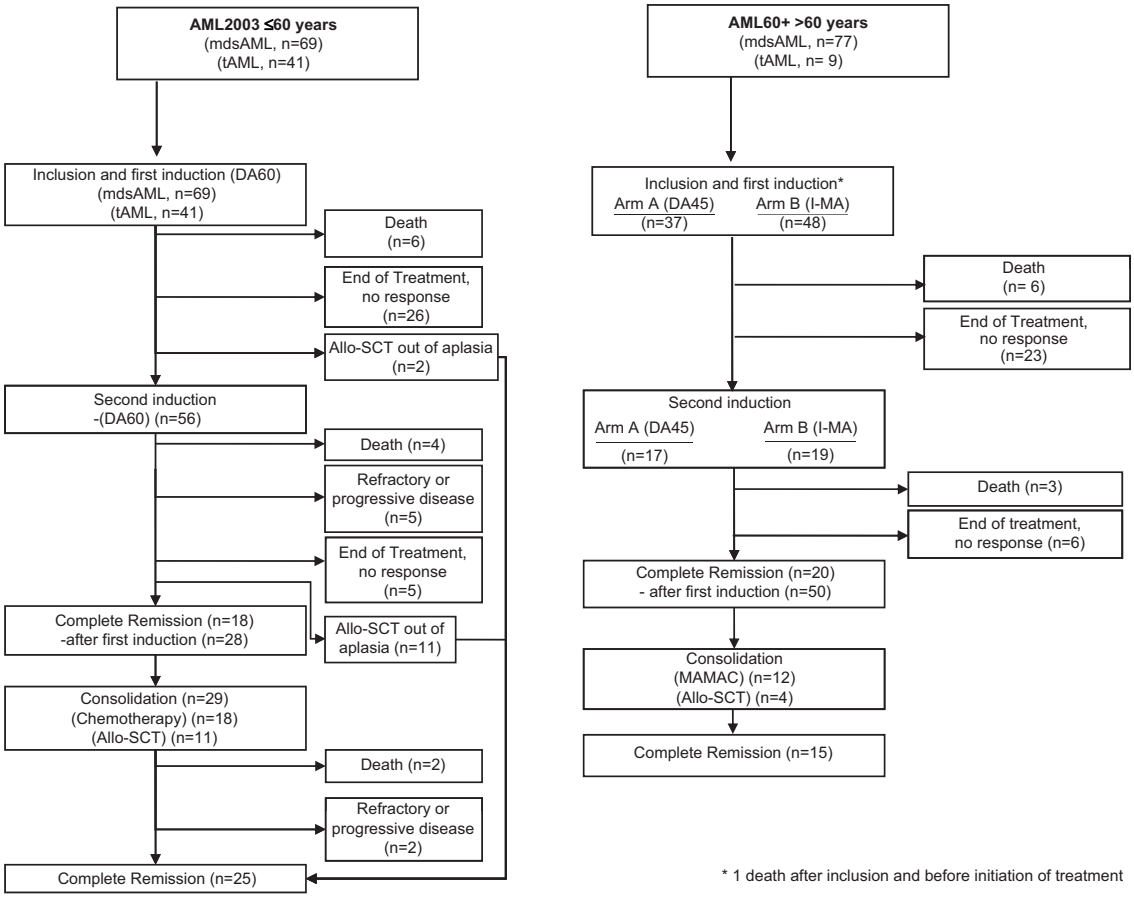

Figure 2 Modified consort flow diagram showing patient numbers, treatments and outcomes of the AML2003 and AML60 + trials.

versus high risk), *NPM1* mutational status, *FLT3-ITD* mutational/wild-type ratio, and bone marrow blast count at day 15 after first induction chemotherapy.

Applying a logistic regression, significant parameters for achieving a CR were: age (OR = 0.38 (95% CI, 0.21–0.67), $P = 0.001$), bone marrow blasts in bone marrow aspirate at day 15 after induction (OR = 0.22 (95% CI, 0.1–0.46), $P < 0.001$), bone marrow $CD34^+$ blasts at diagnosis (OR = 0.33 (95% CI, 0.15–0.69), $P = 0.004$), platelet count in the peripheral blood at diagnosis (OR = 1.97 (95% CI, 1.1–3.52), $P = 0.022$) and *NPM1* mutational status at diagnosis (OR = 4.05 (95% CI, 1.56–10.48), $P = 0.004$). Postremission therapy consisted of chemotherapy ($n = 26$) and/or autologous HSCT ($n = 14$), allogeneic-related HSCT ($n = 8$) and allogeneic-unrelated HSCT ($n = 8$) in all patients with CR after second induction. Postremission therapy was not initiated in 23 patients because relapse occurred before starting. For those patients who underwent consolidation therapy ($n = 56$), there was no significant difference between those patients being treated with chemotherapy and/or autologous HSCT versus those undergoing allogeneic HSCT regarding OS and EFS. Owing to the small number of patients in postremission therapy, adding treatment characteristics to the prognostic factors into the model was impossible. OS and EFS at 2 years for all patients was 20 and 13%, respectively.

Prognostic model for sAML

Prognostic factors for survival were analyzed in the whole group of sAML patients ($n = 305$). In the multivariate Cox model, the dichotomized prognostic factors platelet count in the peripheral blood at diagnosis (HR = 1.95 (95% CI, 1.51–2.5), $P < 0.001$), *NPM1* mutational status in the bone marrow at diagnosis (HR = 1.75 (95% CI, 1.04–2.94), $P = 0.03$), age (HR = 1.37 (95% CI, 1.04–1.81), $P = 0.025$) and cytogenetic risk (HR = 1.15 (95% CI, 1–1.32), $P = 0.04$) were independent prognostic factors for OS in this subgroup of patients. For EFS, the platelet count in the peripheral blood at diagnosis (HR = 1.72 (95% CI, 1.33–2.21), $P < 0.001$), *NPM1* mutational status in the bone marrow at diagnosis (HR = 2.04 (95% CI, 1.21–3.46), $P = 0.008$) and age (HR = 1.5 (95% CI, 1.14–1.98), $P = 0.004$) were independent prognostic factors. Thus, disease status (mdsAML versus tAML) was not an independent prognostic factor for treatment outcome. All of these factors keep their significance when only patients with known *NPM1* status ($n = 213$) were analyzed. However, stratification for age ($\leqslant 60$ and > 60 years) revealed that the *NPM1* status lost its significance in the group aged > 60 years (Table 2).

By assigning a score of one point for each risk factor (age > 60 years, high-risk karyotype, *NPM1* wild type in the bone marrow at diagnosis and a platelet count of $\leqslant 50$ Gpt/l in the peripheral blood at diagnosis), a prognostic score for all sAML patients was created, which summarizes patients harboring 0–1 risk factors in the favorable-risk group, 2 risk factors in the intermediate-risk group and 3–4 risk factors in the high-risk group.

Thus, three risk groups for patients with sAML could be established: the favorable-risk group accounting for 54 patients having a 2-year OS of 52% (95% CI, 38.3–64.9%), the intermediate-risk group with 105 patients having a 2-year OS of 21% (95% CI, 13.5–29.1%) and the high-risk group summarizing 146 patients with sAML having a 2-year OS of only 7% (95% CI, 2.9–11.1%), $P < 0.001$, (Figure 3a).

For the EFS, the distributions were as follows: the favorable-risk group having a 2-year EFS of 44% (95% CI, 30.8–57.4%), the intermediate-risk group having a 2-year EFS of 12% (95% CI, 5.3–17.9%) and the high-risk group having a 2-year EFS of 3% (95% CI, 0–5.4%), $P < 0.001$, (Figure 3c). The results of OS and EFS were comparable between the whole group of patients ($n = 305$) and patients with known *NPM1* status ($n = 213$) (see Supplementary Figures 1a and b).

To validate this prognostic score, the model was applied to an independent sample of patients. Therefore, 196 sAML patients from the prospective AML2003- and and AML60+ trials of the DSIL were assigned to the same scoring system. The clinical characteristics and laboratory data at diagnosis of the 146 patients with mdsAML and 50 patients with tAML from the AML2003− and AML60+ trials are depicted in Table 1. The distribution of patients with mdsAML and tAML was similar to the training set: 47 patients were included in the favorable-risk group, 83 patients in the intermediate-risk group and 66 patients in the high-risk group. The model revealed the same survival differences between the three groups as in the training set: the favorable-risk group having a 2-year OS of 58% (95% CI, 42.3–74.1%), the intermediate-risk group having a 2-year OS of

Table 2 Results of multivariate analysis for OS

	All patients (n = *305*)		*⩽60 years* (n = *115*)		*>60 years* (n = *190*)	
	HR (95% CI)	P-*value for multivariate analysis*	*HR (95% CI)*	P-*value for multivariate analysis*	*HR (95% CI)*	P-*value for multivariate analysis*
Age	1.37 (1.04–1.81)	0.025	—	—	—	—
Disease status (mdsAML vs tAML)	1.04 (0.78–1.4)	0.773	1.2 (0.76–1.9)	0.42	0.95 (0.64–1.4)	0.816
Median WBC count at diagnosis, Gpt/l	1.35 (0.98–1.86)	0.065	1.98 (1.21–3.24)	0.006	1.14 (0.77–1.67)	0.508
Median platelet count at diagnosis, Gpt/l	1.95 (1.51–2.5)	<0.001	3.8 (2.379–6.1)	<0.001	1.43 (1.06–1.94)	0.02
Median serum LDH at diagnosis, U/l	1.41 (1–1.97)	0.047	1.08 (0.57–2.04)	0.818	1.87 (1.29–2.7)	0.001
Median CD34+ bone marrow blasts at diagnosis, %	1.47 (1.01–2.13)	0.043	2.02 (1.13–3.61)	0.017	0.99 (0.6–1.62)	0.969
Cytogenetic risk status (high-risk AML vs standard-risk AML)	1.15 (1–1.32)	0.04	1.22 (0.97–1.55)	0.089	1.09 (0.92–1.28)	0.31
NPM1 mutational status	1.75 (1.04–2.94)	0.03	2.73 (1.26–5.92)	0.011	0.967 (0.51–1.84)	0.918
FLT3 mutational/wild-type ratio	0.9 (0.67–1.21)	0.49	1.2 (0.67–2.14)	0.533	0.98 (0.7–1.36)	0.885
Bone marrow blast count at day 15 after first induction chemotherapy	1.08 (0.79–1.5)	0.618	1.34 (0.77–2.32)	0.305	1.1 (0.74–1.63)	0.064

Abbreviations: AML, acute myeloid leukemia; CI, confidence interval; FLT3, fms-like tyrosine kinase 3; HR, hazard ratio; LDH, lactate dehydrogenase; MDS, myelodysplastic syndrome; mdsAML, AML with preceding MDS; NPM1, nucleophosmin 1; tAML, therapy-related AML; WBC, white blood cells.

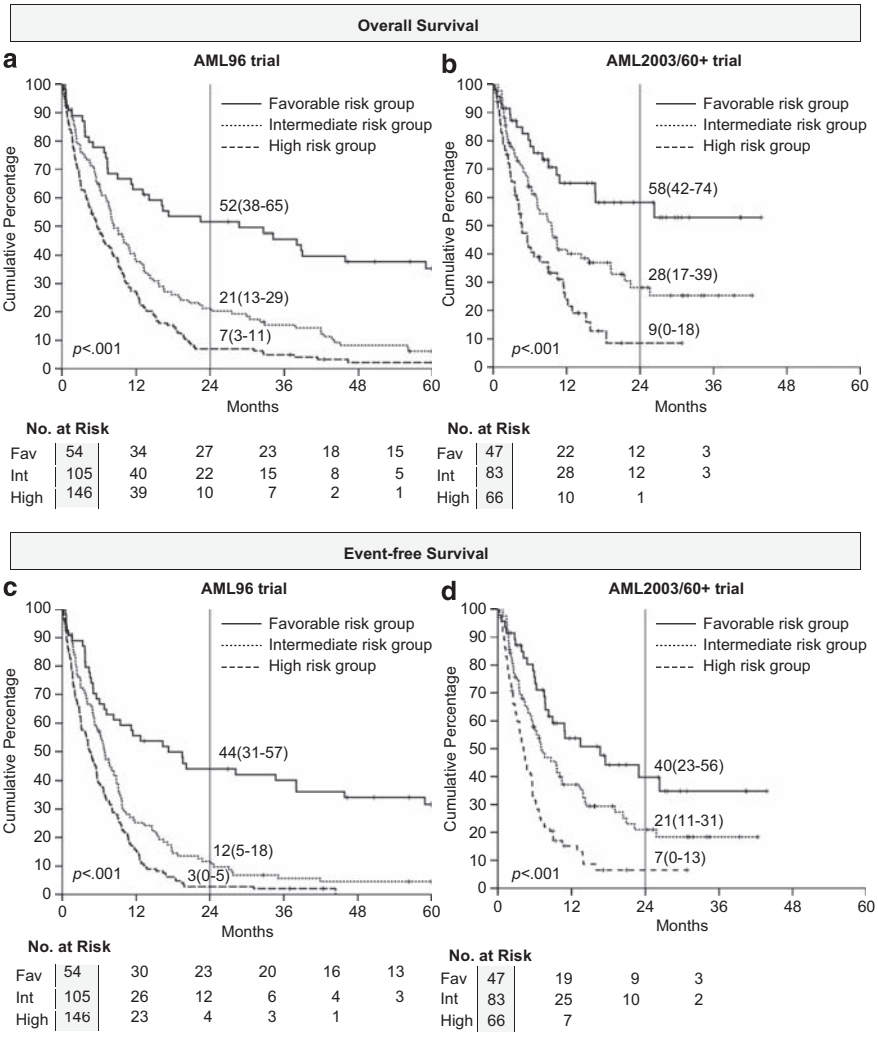

Figure 3 OS and EFS of patients with sAML using the new prognostic model. Patients harboring 0–1 risk factors are summarized in the favorable-risk group, 2 risk factors in the intermediate-risk group and 3–4 risk factors in the high-risk group. (**a**) OS score groups within the AML96 trial. (**b**) OS score groups within the AML2003/60+ trial. (**c**) EFS score groups within the AML96 trial. (**d**) EFS score groups within the AML2003/60+trial. For the patient numbers at risk: Fav, favorable-risk group; Int, intermediate-risk group; High, high-risk group.

28% (95% CI, 16.7–39.5%) and the high-risk group having a 2-year OS of 9% (95% CI, 0–18.1%), $P<0.001$, (Figure 3b). The 2-year EFS in the favorable-risk group was 40% (95% CI, 23.3–56.3%), in the intermediate-risk group, it was 21% (95% CI, 10.8–31.2%) and in the high-risk group it was 7% (95% CI, 0–13.2%), $P<0.001$, (Figure 3d).

Discussion

The presented analysis provides a prognostic scoring system for patients with sAML for the first time. Furthermore, this simple prognostic score has been validated in an independent sample of sAML patients, revealing the same survival differences, although different treatment strategies were applied.

The chosen variables at diagnosis and after first induction therapy, which were evaluated for their potential influence on CR and survival, were selected upon their suggested significant influence, which has been attributed to patients with AML in previous reports: age,[7,16] disease status (mdsAML versus tAML),[7] WBC count,[7,17,18] serum lactate dehydrogenase,[16–18] platelet count,[15] CD34$^+$ bone marrow blasts at diagnosis,[19,20] cytogenetic subgroups,[7,16–17,21] *NPM1* mutational status,[11,22] *FLT3-ITD* mutational/wild-type ratio,[10] and bone marrow blast count at day 15 after first induction chemotherapy.[17]

The absolute platelet count in the peripheral blood and the *NPM1* mutational status in the bone marrow at diagnosis were predictive for OS for patients with sAML. By combining these two new prognostic risk factors for survival with the known predictors, age and karyotype at diagnosis, a prognostic risk group-scoring system was established. The risk factors, age and cytogenetics, have been well described in AML and especially in older AML patients in whom increased frequencies of sAML occur.[7,21]

Platelet counts have repeatedly been reported to be a predictive marker for survival in patients with MDS.[23,24] However, there are data indicating that platelet count levels alone lack additive prognostic information to the International Prognostic Scoring System (IPSS) regarding OS or time into AML evolution.[25] In AML, the impact of the platelet count on OS for adult patients up to 60 years of age harboring aberrations of chromosome band 11q23 has been published recently. In this study, a low platelet count ($\leqslant$50 Gpt/l) was found to negatively impact OS in the whole-patient cohort.[15] However, the impact

of platelet count at diagnosis for patients with sAML, to our knowledge, was outlined in our analysis for the first time. Secondary AML *per se* was shown to be an adverse factor for achievement of CR and for OS.[7,15] An analysis that compared matched tAML- with *de novo* AML patients within another series could not show any difference between these two cohorts by means of CR, disease-free survival (DFS) and OS.[26] Whether these divergent observations are because of different therapy regimens remain unclear. Large reports on age-related risk profiling and chemotherapy dose response in AML that excluded sAML and elderly AML patients including sAML did not include platelet count as a prognostic factor.[7,17] Other studies also indicate that heterogeneity of treatment strategies is higher in elderly AML patients, with sAML becoming an increasing fraction among this cohort, as many patients who are not eligible for clinical trials are not registered and are therefore treated individually.[27,28]

Concerning *NPM1* mutation, enormous efforts have been employed to delineate its relevance.[11,29] The incidence of mutated *NPM1* in tAML in our series matches exactly the incidence in a previously published study.[30] Mutated *NPM1* were found in mdsAML as well as in tAML that supposedly harbor more frequent cytogenetic abnormalities than *de novo* AML.[11,30–32] As *NPM1* mutations seem to occur mainly in *de novo* AML with normal karyotypes, some questions arise concerning their finding in other AML entities: there have been legitimate concerns whether this might indicate that *NPM1*-mutated tAML differs cytogenetically and molecularly from other tAML subtypes or whether those few reported cases of *NPM1*-mutated tAML represents in fact *de novo NPM1*-mutated AML incidentally arising in patients with a history of cytotoxic therapy.[33] Proposed data and models exist, favoring the hypothesis that *NPM1* mutations are a 'primary event' in leukemogenesis[11] or are 'founder genetic alterations', defining a distinct AML entity.[34] Additionally, extensive studies were able to demonstrate that *NPM1* mutations are most relevant in younger AML patients with normal karyotype and less frequent in patients with karyotype abnormalities.[11,29,34–37] Supporting this literature, we found mutation of *NPM1* loses its significant influence on survival for patients older than 60 years of age in the multivariate analysis. As we are aware that all sAML patients in our study population as well as those in our test set were already treated risk adapted for age and cytogenetic risk, which is the international standard for AML therapy, it is difficult to determine whether this finding is an age- or a treatment-related effect. Therefore, we cannot exclude that this might have influenced the outcome regarding our prognostic score. Furthermore, this prognostic score should be validated in a prospective analysis. Future studies might need to address whether the here-described high-risk group of sAML patients might benefit from early reduced intensified conditioning allogeneic HSCT during induction chemotherapy-induced aplasia. With regard to every scoring system, caution must be raised when therapeutic decisions are being made solely by risk group stratification—as daily hospital routine teaches numerous other factors that need to be taken into account for best determining treatment options, with many of them not being applicable for statistical evaluation. Currently, discussions on treatment outcomes of AML patients are largely determined on the basis of genotypic differences.[38] But as the majority of genetic aberrations as well as their altered pathways remain so far not discovered or poorly understood, combined scores comprising clinical data as well as molecular studies might be helpful to stratify patients for different treatment options. However, our model, which combines clinical as well as molecular data in sAML patients, is both easy to obtain and useful for dividing patient subgroups into high-, intermediate- and low-risk subgroups in this AML entity, and displays a prognostic scoring system in sAML throughout different treatment strategy groups for the first time in the literature. Future studies will need to address the question of whether individualized therapy on the basis of this prognostic model will improve treatment results in sAML patients.

Conflict of interest

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on the Leukemia website (http://www.nature.com/leu)

Appendix

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