The Clinical Significance of Diagnostic Red Cell Distribution Width in Patients with Acute Myeloid Leukemia

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Referat:

Introduction: Acute myeloid leukemia (AML) is a highly heterogeneous disease which renders risk stratification at diagnosis of high importance to personalize therapy. Allogeneic hematopoietic stem cell transplantation (HSCT) offers the highest chance for sustained remission in most AML patients, but usually comes at the risk of a significant treatment-related mortality. The red cell distribution width (RDW) is an universally accessible parameter that identifies individuals with a higher mortality in many diseases, including some hematological entities. However, the impact of diagnostic RDW levels in AML – especially in the context of a HSCT consolidation - has not been evaluated so far.

Purpose: To evaluate the prognostic impact of RDW levels at AML diagnosis.

Methods: A total of 294 newly diagnosed AML patients (median age 60.6, range 14.3-76.5 years), with available diagnostic RDW levels were retrospectively included in this analysis. All patients received a consolidation therapy with an allogeneic HSCT in curative intention between August 2007 and December 2020 at the University Medical Center Leipzig. The RDW was measured in all patients at AML diagnosis before the start of cytoreductive therapies.

Results: RDW levels at diagnosis were highly variable (median 16.6%, range 12%-30.6%) and above the upper level of normal (>15%) in 73% of the analyzed AML patients. Patients with RDW levels above 15% did not have worse outcomes compared to patients with low diagnostic RDW levels. However, when the cohort was dichotomized according to a receiver operating characteristic (ROC)-based optimal cut-point (20.7%), patients with high RDW levels had a significantly higher non-relapse mortality (NRM), shorter overall survival and a trend for shorter event-free survival, while the risk of relapse or disease progression was similar in both groups. In multivariate analyses, the RDW remained an independent prognostic factor for higher NRM after adjustment for the body mass index at diagnosis. Patients with a higher RDW were more likely to harbor a secondary AML, as well as to harbor secondary AML-associated gene mutations (*i.e. JAK2, ASXL1*, or spliceosome mutations, especially *SRSF2*).

Conclusion: High RDW levels at diagnosis represent an independent risk marker for a higher mortality following allogeneic HSCT. When confirmed in prospective clinical trials, the RDW might help to personalize AML consolidation therapy including conditioning regimens before allogeneic HSCT.

2. Abkürzungsverzeichnis

ALL	acute lymphoblastic leukemia
AML	acute myeloid leukemia
AML-MRC	acute myeloid leukemia with myelodysplasia related changes
APL	acute promyelocytic leukemia
ASXL1	additional sex combs-like 1
ATG	anti thymocyte globulin
BAALC	brain and acute leukemia, cytoplasmic
BMI	body mass index
CBF	core binding factor
CEBPA	CCAAT/enhancer-binding protein alpha
CHIP	clonal hematopoesis of indeterminate origin
CI	confidence interval
CIBMTR	Center for International Blood and Marrow Transplant Research
CIR	cumulative incidence of relapse
CML	chronic myeloid leukemia
CMV	cytomegalovirus
CR	complete remission
CRi	complete remission with incomplete peripheral recovery
DNA	deoxyribonucleic acid
DNMT3A	DNA methyltranspherase 3 alpha
ECOG	Eastern Cooperative Oncology Group
EDTA	ethylenediaminetetraacetic acid
EFS	event-free survival
ELN	European LeukemiaNet
FISH	fluorescent *in situ* hybridization

FLAMSA	fludarabine, amsacrine, and cytarabine
FLT3-ITD	Fms-related tyrosinkinase-3-gene – internal tandem duplication
FLT3-TKD	Fms-related tyrosinkinase-3-gene – tyrosine kinase domain
GO	gemtuzumab ozogamicin
Gpt/l	giga particle per liter
GvHD	graft-versus-host disease
GvL	graft-versus-leukemia
Gy	Gray
HCT-CI	hematopoietic cell transplantation-specific comorbidity index
HLA	human leukocyte antigen
HR	hazard ratio
HSCT	hematopoetic stem cell transplantation
IDH1	isocitrate dehydrogenase 1
IDH2	isocitrate dehydrogenase 2
JAK2	janus kinase 2
LDH	lactate dehydrogenase
LSCs	leukemia stem cells
MAC	myeloablative conditioning
MDS	myelodysplastic syndrome
MDS/MPN	myelodysplastic/myeloproliferative neoplasm
MN1	meningeoma-1
MPN	myeloproliferative neoplasm
MRC	Medical Research Council
MRD	measurable residual disease
NGS	next generation sequencing
NHL	non-hodgkin lymphoma

NMA	non-myeloablative
NPM1	nucleophosmin 1
NRM	non-relapse mortality
OR	odds ratio
OS	overall survival
OSHO	Ostdeutsche Studiengruppe für Hämatologie und Onkologie
PBS	phosphate buffered saline
PCR	polymerase chain reaction
R-CHOP	Rituximab, Cyclophosphamide, Hydroxirubicin (Adriamycin), Oncovin (Vincristin), Prednisone
RDW	red cell distribution width
RIC	reduced intensity conditioning
ROC	receiver operator characteristic
RUNX1	runt-related transcription factor 1
SF3B1	splicing factor 3b subunit 1
SRSF2	serine and arginine rich splicing factor 2
TBI	total body irradiation
TP53	tumor protein 53
U2AF1	U2 small nuclear RNA auxiliary factor 1
WHO	World Health Organization
WT1	Wilms tumor 1
ZRSR2	zinc finger (CCCH type), RNA-binding motif and serine/arginine rich 2

3. Einführung / Introduction

3.1. Acute myeloid leukemia

3.1.1. Definition

Acute myeloid leukemia (AML) is a heterogenous malignant hematological disease characterized by an increase and clonal expansion of myeloid precursors in blood, bone marrow, or other tissues.[1] The pathophysiology of AML is based on the genetic alterations of hematopoietic progenitor cells, so called leukemic stem cells (LSCs), resulting in the clonal proliferation of immature, dysfunctional myeloblasts. This dysfunctionality manifests by failure to differentiate and to die by the process of apoptosis.[2,3]

3.1.2. Epidemiology and etiology

AML is a disease of older age with an annual incidence in the United States of 2.4 cases per 100,000 individuals.[4] The incidence is further increasing with older age to peak at 12.6 per 100,000 in patients aged 65 years or older.[4]

AML can occur "*de novo*", or as a secondary neoplasm, either developing secondary after related hematological malignancies such as myelodysplastic syndrome (MDS) or myeloproliferative neoplasm (MPN),[5] or treatment-related, *i.e.* after prior exposure to chemotherapy or radiation, or after non-therapeutic toxic exposures. Typical cytotoxic substances known to predispose to developing AML include alkylating agents (such as thiotepa or nitrogen-mustard) after a median of 5-7 years, and topoisomerase-inhibitors, after a median of 2-3 years.[6,7]

Potential occupational and environmental DNA-damaging agents which can contribute to leukemogenesis include benzene, an aromatic hydrocarbon, commonly used as a solvent in the industry of plastic, rubbers, and lubricants.[6]

More rarely, AML may also develop in individuals with congenital or germline predispositions.[8] One of the recently defined premalignant conditions to MDS and AML is clonal hematopoiesis of indeterminate potential (CHIP).[9] CHIP is defined as the presence of at least one somatic mutation in a leukemia associated driver gene with a variable allele frequency of ≥2% in peripheral blood without

detectable cytopenia or dysplasia, and exclusion of a hematological disease as an underlying condition.[10]

3.1.3. Clinical presentation

Patients' clinical presentation at AML diagnosis is heterogenous, but in most cases a consequence of the increasing bone marrow infiltration by myeloid blasts, resulting in various signs of cytopenia. The patients may present with pallor, fatigue, or an impaired performance ability as a result of anemia and petechia or other bleeding stigmata as a result of thrombocytopenia. The loss of mature functional granulocytes and lymphocytes may result in a variety of bacterial or viral infections. Occasionally, the accumulation of leukemic blasts can cause gingival hyperplasia – which typically accompanies monoblastic/monocytic AML and hyperleukocytosis, swellings of lymph nodes or hepatosplenomegaly. In very rare cases, leukemic blasts may also accumulate as tumor in other organs, which is referred to as chloroma or myeloid sarcoma when no elevated blast counts in blood or marrow are present.[4,11]

Extreme leukocytosis can induce the life-threatening symptoms of leukostasis. Furthermore, the associated metabolic changes like hyperuricemia or hypocalcemia may be seen at presentation.

3.1.4. Diagnosis of AML

3.1.4.1. Morphology

The primary diagnosis of AML is based on the morphologic identification of myeloid blasts in blood or bone marrow aspirates after staining with Wright-Giemsa.[4] The blood smears are evaluated by counting at least 200 white blood cells and bone marrow smears by counting at least 500 white blood cells. According to the classification of the World Health Organization (WHO) the diagnostic criterion for AML is the finding of at least 20% myeloid blasts in peripheral blood or bone marrow.[12] An exception represents the presence of specific recurrent chromosomal aberrations, *i.e.* t(15;17)(q22;q12) (resulting in the fusion gene *PML-RARA*), t(8;21)(q22;q22) (resulting in the fusion

gene *RUNX1-RUNX1T1*), and inv(16)(p13q22) or its variant t(16;16)(p13;q22) (both resulting in the fusion gene *CBFB-MYH11*). Here, the cytogenetic irrespective of the blast count determines the diagnosis of AML.

The further identification of differentiation markers – important for not otherwise specified AML or the distinction from other hematologic neoplasm - may be performed by cytochemistry or immunophenotyping.

3.1.4.2. Immunophenotyping

The most common immunophenotyping method for determination of differentiation features in AML is flow cytometry. Flow cytometry allows the analysis of the presence or absence of specific antigens on an individual cell in a suspension[13] and to distinguish AML from acute lymphoblastic leukemia (ALL) by demonstration of myeloid lineage markers.[12,14–16] Furthermore, flow cytometry is a powerful tool for determination of the fraction of LSC. LSCs are a small population of malignant cells, which are more therapy resistant than the bulk of leukemia cells, and often initiate relapse after the applied treatments. Those LSCs may express different immunophenotypes, such as CD34+/CD38+, and CD34, but the majority has been shown to exist within the CD34+/CD38- cell population.[17,18] The CD34+/CD38- population seem to be the least immunogenic and the one most refractory to the applied treatments.[19,20]

The rare type of acute leukemia of ambiguous lineage can also be diagnosed per immunphenotyping.[21] It comprises acute undifferentiated leukemia and mixed phenotype leukemia. Acute undifferentiated leukemia lack the expression of markers such as cytoplasmatic CD3, MPO, CD19, cytoplasmatic CD22, and CD79, typically expresses only one of the surface lineage markers CD13, CD33, or CD7, with weak or partial positive expression.[22] The mixed phenotype leukemia, on the other hand, is further subdivided into biphenotypic leukemia, where one blast population expresses both myeloid and lymphoid markers and mixed phenotype leukemia, with two or more single lineage leukemia populations.[22,23]

3.1.4.3. Cytogenetic and molecular analyses

Over recent years, the importance of genetic characterization became increasingly evident. Today, they are obligatory examinations in every patient with newly diagnosed AML, as they provide important information on risk stratification and guide informed treatment decisions. More than 55% of the patients diagnosed with AML show chromosomal abnormalities.[24] At diagnosis, a minimum of 20 metaphases are mandatory to perform standard banding techniques to determine the karyotype of the disease.[24,25] Fluorescence *in situ* hybridization (FISH) may complement standard karyotyping by identifying or excluding specific aberrations, especially in cases where no metaphases can be obtained.[26]

Depending on the number of analyzed genes, nearly all individuals diagnosed with AML show at least one molecular aberration,[27] which can be detected by polymerase chain reaction (PCR)- or targeted next generation sequencing (NGS)- based methods. Some have already been included into risk stratification systems, which are most likely to be further developed in the coming years, *e.g.* with genome wide studies.[28]

3.1.5. AML classification according to WHO classification

The WHO classification is the most commonly used classification system for AML.[12,16] With the last update in 2016, the growing number of recurrent genetic changes and their prognostic relevance in AML is accounted for with the introduction and/or confirmation of distinct genetic entities.[16]

In contrast to the other listed categories, the category myeloid sarcoma is a unique clinical presentation of any subtype of AML. Although listed as a separate category, patients with myeloid sarcoma present without evidence of involvement of blood or bone marrow and should be further investigated in order to be classified into a more specific subtype of AML.[16] Table 1 gives an overview of the last update from 2016 of the WHO classification of AML.

Table 1: Adapted from Arber *et al*:[16] The 2016 revision of the WHO classification of AML

Acute myeloid leukemia and related neoplasms
Acute myeloid leukemia with recurrent genetic abnormalities
AML with t(8;21)(q22;q22.1); *RUNX1-RUNX1T1*
AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*
APL with *PML-RARA*
AML with t(9;11)(p21.3;q23.3); *MLLT3-KMT2A*
AML with t(6;9)(p23;q34.1); *DEK-NUP214*
AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); *GATA2, MECOM*
AML (megakaryoblastic) with t(1;22)(p13.3;q13.3); *RBM15-MKL1*
Provisional entity: AML with BCR-ABL1
AML with mutated *NPM1*
AML with biallelic mutations of *CEBPA*
Provisional entity: AML with mutated RUNX1
Acute myeloid leukemia with myelodysplasia-related changes
Therapy-related myeloid neoplasms
Acute myeloid leukemia, not otherwise specified
AML with minimal differentiation
AML without maturation
AML with maturation
Acute myelomonocytic leukemia
Acute monoblastic/monocytic leukemia
Pure erythroid leukemia
Acute megakaryoblastic leukemia
Acute basophilic leukemia
Acute panmyelosis with myelofibrosis
Myeloid sarcoma
Myeloid proliferations related to Down syndrome
Transient abnormal myelopoiesis
Myeloid leukemia associated with Down syndrome

3.1.6. Prognostic factors in AML

3.1.6.1. Patient-related risk factors

Since AML is a highly heterogeneous disease, much effort has been put into identifying clinical and genetic risk factors to facilitate risk stratification. Adverse clinical factors include older - either chronological or biological – age, impaired performance status (typically assessed by the ECOG system),[29] and the presence of comorbidities, as all may prevent intensive treatment approaches. Also elevated lactate dehydrogenase (LDH) levels and a higher body mass index (BMI) at presentation have been suggested as adverse risk factors in AML.[4,30] At least in the context of chemotherapy consolidation, also secondary and treatment-related AML associate with adverse treatment outcomes compared to *de novo* disease.[31,32]

3.1.6.2. Genetic risk factors

Over time, the critical prognostic information provided by chromosomal abnormalities at presentation became increasingly evident. The prognostic relevance of the karyotype of myeloid blasts was shown in 1998 in a large patient population trial of the Medical Research Council (MRC)[25] and was successively confirmed by other study groups.[33,34]

Approximately 20% of younger and less than 10% of older AML patients show a favorable karyotype at presentation. Favorable cytogenetic aberrations include the acute promyelocytic leukemia [APL, t(15;17)(q22;q12)], as well as the core-binding factor AMLs [CBF, t(8;21)(q22;q22) or inv16(p13;q22)].[4,35,36] On the other hand, approximately 15% of the patients show adverse cytogenetic aberrations, which are enriched in secondary or treatment-related AML and older individuals.[37] Typical chromosomal abnormalities include monosomies of chromosomes 5 or 7, the deletion of the long arm of 5 (del5q), abnormalities of chromosome 17, the long arm of chromosome 3 or three or more genetic aberrations in one clone, referred to as a complex karyotype.[4,35,36] Also the presence of one somatic monosomy together with a second genetic aberration (referred to as monosomal karyotype) has been shown to predict inferior outcomes.[38]

Using only cytogenetic risk stratification, the majority of patients has to be classified in a standard-risk group showing either a normal karyotype or cytogenetic abnormalities not included in the other groups.[39] However, the treatment responses and long-term survival of this standard-risk group remains highly heterogeneous. To refine the risk profile of these standard-risk patients, mutations in the genes nucleophosmin 1 (*NPM1),* and CCAAT/enhancer-binding protein alpha (*CEBPA*), as well as internal tandem duplications in the *FLT3* gene (*FLT3*-ITD), which allow additional risk stratification, were identified. The European LeukemiaNet (ELN) was the first organization to propose a risk stratification system in AML in 2010, that also included molecular alterations. Later, a variety of groups independently validated the prognostic relevance, also independently of the applied consolidation therapies.[40–42]

In 2016, the ELN published the first updated version of the genetic risk categories.[43] This current ELN risk stratification now distinguishes between three risk groups: favorable, intermediate, and adverse. Patients with CBF AML, biallelic mutated *CEBPA* and either mutated *NPM1* with absent *FLT3*-ITD or low *FLT3*-ITD allelic ratio (<0.5 mutant to wild-type ratio) are now assigned into the favorable risk group. As the presence of additional chromosomal aberrations in patients with *NPM1* or biallelic *CEBPA* mutations did not modify the prognostic favorable effect of the mutations,[44–46] those patients are categorized as favorable risk irrespective of karyotype. Patients with mutations in the genes *RUNX1*, *ASXL1*, and *TP53* were added to the cytogenetically characterized adverse risk group.[47] Also AML patients with wild-type *NPM1* and high allelic ratio of *FLT3*-ITD have poor prognosis and are categorized in the adverse group.[48] The mutations in other potentially important genes such as *DNMT3A*, *IDH1*, *IDH2*, or genes associated with the splicing apparatus were not further categorized, as data seemed insufficient to draw final conclusion.[47] Table 2 depicts the genetic risk groups according to the current ELN2017 classification system.

Table 2. (Adapted from Döhner *et al.*):[47] 2017 European LeukemiaNet genetic risk stratification system[a]

Risk Category[b]	Genetic Abnormality
Favorable	t(8;21)(q22;q22); *RUNX1-RUNX1T1* inv(16)(p13.1q22) or t(16 ;16)(p13.1;q22); *CBFB-MYH11* mutated *NPM1* without *FLT3*-ITD or with *FLT3*-ITD$^{low\ l}$ biallelic mutated *CEBPA*
Intermediate	mutated *NPM1* and *FLT3*-ITD$^{high\ l}$ wild-type *NPM1* without *FLT3*-ITD or *FLT3*-ITD$^{low\ l}$ (without adverse-risk genetic lesions) t(9;11)(p22;q23); *MLLT3-KMT2A*[d] cytogenetic abnormalities not classified as favorable or adverse
Adverse	t(6;9)(p23;q34.1); *DEK-NUP214* t(v;11q23.3); *KMT2A* rearranged t(9;22)(q34.1;q11.2); *BCR-ABL1* inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); *GATA2*, *MECOM* (*EVI1*) -5 or del(5q); -7; -17/abn(17p) complex karyotype,[e] monosomal karyotype[f] wild-type *NPM1* and *FLT3*-ITD^{high} mutated *RUNX1*[g] mutated *ASXL1*[g] mutated *TP53*[h]

[a] Frequencies, response rates and outcome measures should be reported by risk category, and, if sufficient numbers are available, by specific genetic lesions indicated.

[b] Prognostic impact of a marker is treatment-dependent and may change with new therapies.

[c] Low, low allelic ratio (<0.5); high, high allelic ratio (>0.5); semi-quantitative assessment of *FLT3*-ITD allelic ratio (using DNA fragment analysis) is determined as ratio of the area under the curve (AUC) "*FLT3*-ITD" divided by AUC "*FLT3*-wild type"; recent studies indicate that acute myeloid leukemia with *NPM1* mutation and *FLT3*-ITD low allelic ratio may also have a more favorable prognosis and patients should not routinely be assigned to allogeneic hematopoietic-cell transplantation.

[d] The presence of t(9;11)(p21.3;q23.3) takes precedence over rare, concurrent adverse-risk gene mutations.

[e] Three or more unrelated chromosome abnormalities in the absence of one of the World Health Organization-designated recurring translocations or inversions, *i.e.*, t(8;21), inv(16) or t(16;16), t(9;11), t(v;11)(v;q23.3), t(6;9), inv(3) or t(3;3); AML with *BCR-ABL1*.

[f] Defined by the presence of one single monosomy (excluding loss of X or Y) in association with at least one additional monosomy or structural chromosome abnormality (excluding core-binding factor AML).

[g] These markers should not be used as an adverse prognostic marker if they co-occur with favorable-risk AML subtypes.

[h] *TP53* mutations are significantly associated with AML with complex and monosomal karyotype.

3.1.6.3 Measurable residual disease

Whereas the conventional morphological examination can discriminate approximately one leukemic cell in 20 white blood cells, the evaluation of so-called measurable residual disease (MRD) allows a much more sensitive detection of malignant cells with up to one leukemic cell in 10^4-10^6 of total white blood cells. The most common diagnostic methods used for MRD determination are multicolor flow

cytometry and quantitative PCR. The MRD is of special importance during and after treatment in curative intention where it can be used to describe the depth of the remission status, but also to identify patients at high risk of relapse and thus enable an early intervention.[49] According to the ELN recommendations 2017 the determination of MRD should be included in routine clinical care. Furthermore, the ELN2017 defines complete remission (CR) with or without MRD.[47] There is increasing evidence that the MRD status prior to allogeneic HSCT is an independent factor influencing the prognosis. Araki *et al.*, for example showed that the outcomes of patients who underwent allogeneic HSCT with active disease did not differ from that of patients with a positive MRD status at HSCT. On the other hand, patients with negative MRD showed significantly superior outcomes.[50]

3.1.7. Treatment of AML

3.1.7.1 Induction therapy in curative intention

Due to the mostly older age and comorbidities of AML patients at presentation, it is necessary to define realistic treatment goals after having established the diagnosis. The intensive chemotherapy approaches in curative intention are reserved for younger patients without significant comorbidities. On the other hand, older, and/or comorbid individuals are treated in palliative intention aiming for the best achievable quality of life.[4]

The first goal of AML treatment is the induction of a CR, which is defined as less than 5% blast cells in a cellular bone marrow for at least 28 days, with a peripheral neutrophil count of $>1.0 \times 10^9$ Gpt/l, platelet count of at least 100 Gpt/l and the absence of Auer rods or extramedullary disease.[47] The backbone of the standard induction therapy is the combination of seven days of cytarabine with 3 days of an anthracycline, usually daunorubicin, which is referred to as standard “7+3” therapy. With intensive induction treatment 60-80% of patients aged 60 years and younger and 40-60% of patients older than 60 years will achieve a CR.[1,28] Clinical experience has demonstrated that further intensive post-induction treatment is necessary to consolidate the CR and achieve long-term remissions.

3.1.7.2. Consolidation therapies

The post remission strategies include conventional chemotherapy or allogeneic HSCT. The intensive chemotherapy consolidation regimen include intermediate to high-dose cytarabine monotherapy or repeated cytarabine/anthrazycline combination therapy with similar outcome of both approaches.[51] This consolidation option is usually offered to favorable or intermediate-risk patients with good treatment responses. In contrast, adverse risk patients according to ELN2017 classification, patients with suboptimal treatment responses and all patients after first relapse benefit from consolidation therapy with an allogeneic HSCT.

3.1.7.3. Palliative treatment approaches

The approaches for older and/or significantly comorbid AML patients are limited to best supportive care, and low intensity treatment, *i.e.* low dose cytarabine or hypomethylating agents. The treatment with low dose cytarabine is well tolerated, and although being superior to best supportive care, remain unsatisfactory with a median OS of only about five months.[52]

The hypomethylating agents decitabine and 5-azacitidine both showed superiority in treatment responses compared to low-dose cytarabine. In a phase III trial 5-azacitidine achieved a superior median OS of 24.5 months *vs* 15 months for the standard of care with comparable tolerability.[53] Also decitabine was compared to standard of care in a phase III trial showing a superior median OS of 7.7 months compared to 5 months for the standard of care group.[54] Subsequently, hypomethylating agents are the current standard of care to treat AML patients not eligible for intensive induction therapies.

3.1.7.4. New substances

In the last years, a variety of new drugs have been approved for the induction treatment of AML patients. Midostaurin (PKC412) is an oral multitarget kinase inhibitor. In the recently published RATIFY trial, newly diagnosed AML patients received induction and consolidation therapy followed by midostaurin or placebo, as well as maintenance therapy with midostaurin or placebo for up to one year. The group treated with midostaurin showed superiority in terms of CR rate, overall survival (OS) and event-free survival (EFS).[55] Subsequently, midostaurin in combination with intensive chemotherapy is now approved and the new standard of care for treatment of *FLT3*-mutated AML.

Gemtuzumab ozogamicine (GO), an anti-CD33 antibody conjugate with N-acetyl gamma calicheamicin[56] has shown superiority in combination with intensive induction in patients with CD33+ AML.[57] In a recently published meta-analysis, CD33+ AML patients with favorable or intermediate risk had the highest benefit after the treatment with GO, while no benefit was observed for adverse risk patients.[58] Subsequently, while GO is approved for treatment of CD33+ AML irrespective of disease risk, it is generally administered to patients with favorable or intermediate genetic risk according to ELN2017.

CPX-351 is a dual drug liposomal encapsulation of cytarabine and daunorubicin. In a phase III trial CPX-351 was applied in older patients with treatment-related AML or AML with myelodysplasia related changes (AML-MRC) showing higher CR rates and survival benefit compared to standard "7+3" chemotherapy.[59] CPX-351 is approved for induction treatment of therapy-related AML and AML-MRC.

Also for the older and/or comorbid patient cohort, promising treatment option have been recently published. In a phase III trial the combination of 5-azacitidine with venetoclax showed impressive rates of combined CR and CR with incomplete hematological recovery (CRi) of 66% *vs* 28% in the 5-azacitidine placebo group. With 14.6 months, the median duration of OS was superior to 9.6 months in the placebo arm.[60] Upon approval in Europe, the combination of venetoclax with hypomethylating agents is expected to be the new treatment standard for most older AML patients.

3.2. Allogeneic HSCT

3.2.1. Principles of allogeneic HSCT

HSCT refers to any procedure that transfers stem cells of any kind of a donor in order to partially or completely repopulate and replace the hematopoietic system of the patient. Stem cells can be collected from bone marrow, peripheral blood, or cord blood.[61] Today, peripheral blood stem cells represent the most frequently used stem cell source. They are associated with a faster reconstitution but also an increased incidence of chronic graft-versus-host disease (GvHD), which is also regarded as a surrogate marker for the graft-versus leukemia (GvL) effect. Thus, peripheral blood stem cells are the most used graft source in patients with hematologic malignancies, including AML.[62] AML represents the most frequent indication for allogeneic HSCT with a continuously increasing number of transplants performed worldwide.[63]

The decision to perform allogeneic HSCT is depending on the individual risk-benefit ratio for the patient, especially in terms of non-relapse mortality (NRM) and morbidity *vs* relapse risk, and is based on disease risk, as well as patient-related factors, and the availability of a stem cell donor.[62] In patients with adverse-risk AML a higher risk of NRM can be accepted. In primary refractory patients, allogeneic HSCT is the only curative treatment option.[47]

There are following categories of allogeneic donor types: syngeneic, human leukocyte antigene (HLA)-identical sibling donor, other family donor or unrelated donor. The definition of a well-matched unrelated donor is defined as 10/10 or 8/8 matches, based on high resolution typing for class I (HLA-A, -B, -C) and II (HLA-DRB1, -DQB1) antigens, whereas a mismatched unrelated donor is referred to mismatch in at least one antigen or allele at HLA-A, -B, -C or -DR.[64] A family member with only one genetically identical HLA-haplotype is termed as haplo-identical donor. Prior to the administration of the donor stem cells, the patient is treated with conditioning therapy, a combination of chemotherapy drugs and/or radiotherapy. The objectives of conditioning are tumor debulking as well as the prevention of graft rejection.

3.2.2. Conditioning regimens

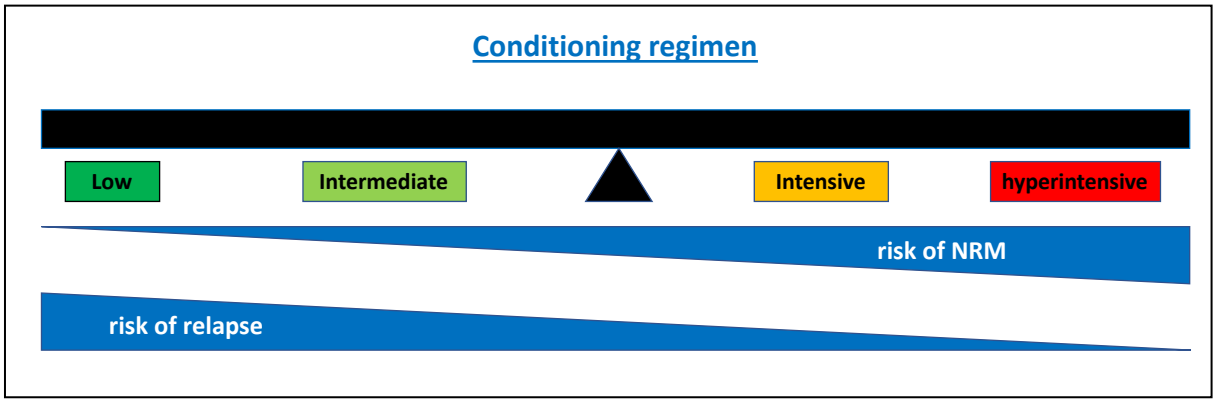
The experts from the Centre for International Blood and Marrow Transplant Research (CIBMTR)[65] classified the conditioning regimens in three groups:

- Myeloablative conditioning (MAC): This conditioning causes a severe myelotoxicity followed by irreversible cytopenias. The application of stem cells is crucial for the patients' survival.
- Reduced-intensity conditioning (RIC): This conditioning also causes significant cytopenias, but includes substantially reduced dosages of radiation or chemotherapy. Autologous regeneration may still be possible.
- Non-myeloablative conditioning (NMA): This is the conditioning with the lowest possible toxicity and causes only mild and transient cytopenias. Autologous regeneration is usually possible.

Whereas the objective of MAC-HSCT is the complete eradication of donor hematopoiesis, the NMA conditioning is based on immunological mechanisms induced by a GvL effect. This recognition is based on the observations from 1980s that patients who developed acute or chronic GvHD after allogeneic HSCT had improved relapse-free survival.[66]

Both MAC and RIC regimens are based on application of alkylating agents with or without total body irradiation (TBI). Compared to MAC, the RIC regimen are usually >30% dose-reduced, thus, defining the intensity on the grade of reversible and irreversible myelotoxicity.[67] It is important to emphasize that individual RIC protocols can vary substantially in their intensity and toxicity.[67] Figure 1 shows an overview of the different intensities and their respective risk of relapse or NRM.

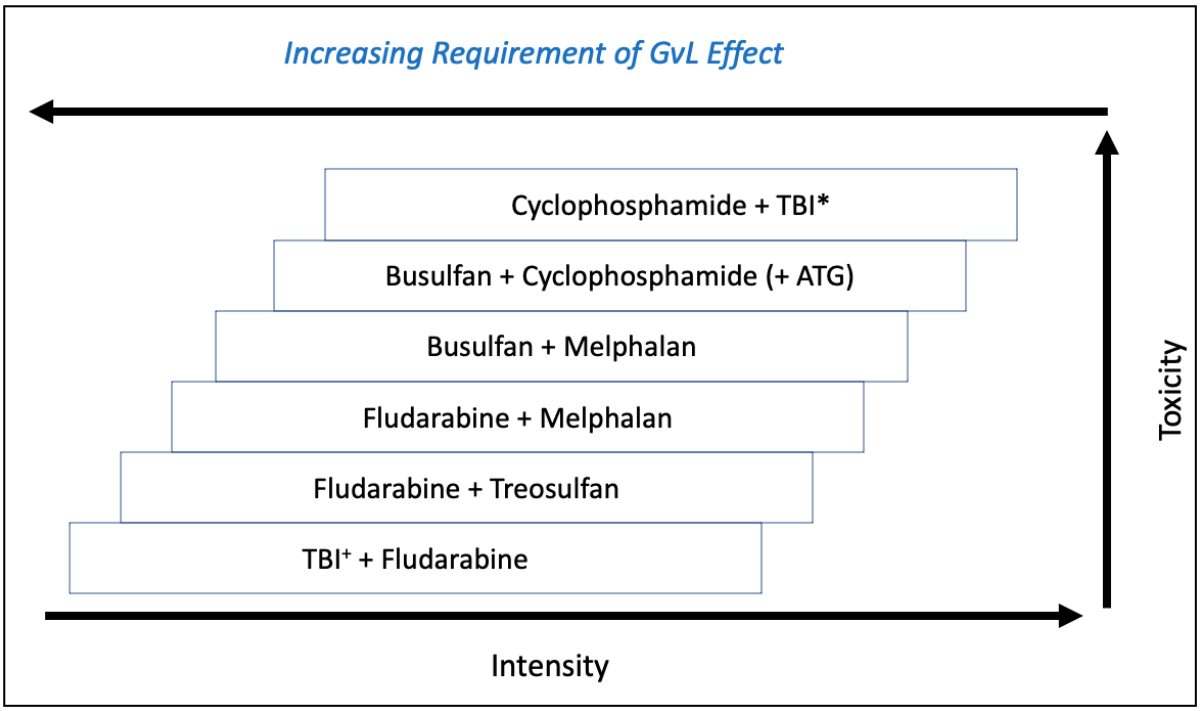
Figure 1: Adapted from Gagelmann *et al*.[67]: The balance between risk for NRM and relapse when choosing conditioning intensity



The remarkable success of RIC and NMA conditioning regimens in the last years resulted in dramatic increase of transplantations worldwide, thus making this option available for patients in whom the conventional consolidation chemotherapy would unlikely be able to provide long-term outcomes. The risk of NRM can be predicted by a variety of scoring systems. One of the most frequently used systems to assess the risk of overall mortality and especially NRM in patients planned for allogeneic HSCT is the hematopoietic cell transplant comorbidity index (HCT-CI).[68]

For each dose intensity protocol, specific examples that were given to the patients included in this study are presented in Figure 2.

Figure 2: Adapted from Gyurkocza *et al.*[69]: Selected conditioning regimens of different dose intensities included in this study.



Legend: ATG, anti-thymocyte globulin; TBI, total body irradiation.
** High-dose total body irradiation (800-1320 cGy), + Low dose total body irradiation (200-400cGy)*[70]

3.3. Red cell distribution width

The red cell distribution width (RDW) reflects the distribution of the red blood cell volume in an individual, and today, is widely reported within the complete blood count. Erythrocytes in general decrease in cellular volume across their lifespan, which is why *e.g*. a delayed clearance of these corpuscles leads to higher RDW levels.[71] A higher RDW is basically a marker for a dysregulated erythrocyte homeostasis and the index generally increases with age. Traditionally, the clinical meaning of the RDW was limited to the differential diagnosis of anemia.[72] Today, however, a higher RDW is also a risk marker for morbidity and mortality in otherwise healthy people, but also in various diseases, and has been linked to oxidative stress and poor nutritional status.[73,74] It also has been suggested that a high RDW defines a pro-inflammatory state,[75] leading to a higher incidence of several cardiovascular

diseases – including atrial fibrillation, heart failure, coronary heart disease, and cardiac mortality as well as a higher likelihood to develop a variety of malignancies.[76–79] Linked to the described pro-inflammatory state[75] is also a higher mortality in acute respiratory distress syndrome patients with a higher RDW.[80] Also in COVID-19 patients, higher RDW levels associated with a worse disease course and a higher mortality.[81,82]

In terms of malignant diseases, the RDW is increasingly recognized to have a prognostic role in carcinogenesis and tumor progression.[83] This is also true for different hematological malignancies. As an example, the RDW may help in assessing the prognosis in patients with diffuse large B-cell lymphoma treated with R-CHOP.[84] Furthermore, a previous study showed that healthy individuals with detectable somatic mutations – which are known to confer a higher risk of developing a myeloid neoplasm – show higher RDW levels than unmutated patients.[85] In individuals with unexplained cytopenias, a high RDW was an independent factor that predicted the diagnosis of MDS[86,87] and to be able to discriminate healthy blood samples from that of MDS patients.[88] A higher RDW might reflect dyserythropoiesis in MDS.[87]

Interestingly, in healthy individuals a higher RDW associates with a higher risk of developing AML, already several years before diagnosis.[76,89] However, in AML, to our knowledge the impact of the diagnostic RDW has not yet been systematically studied.

4. Aufgabenstellung / Objectives

The objectives of the here presented study were

- To assess the distribution of RDW levels in newly diagnosed AML patients.
- To evaluate the prognostic significance of RDW levels at diagnosis in AML patients consolidated with an allogeneic HSCT.
- To evaluate whether RDW levels at AML diagnosis provide prognostic information independently from other relevant prognostic factors in AML.
- To evaluate associations of RDW levels at AML diagnosis with other clinically, genetically, and prognostically relevant factors in AML.

5. Materialien und Methoden / Materials and Methods

5.1. Patients and treatments

5.1.1. Treatment protocols

A total of 294 newly diagnosed AML patients with a median age at diagnosis of 60.6 (range 14.3-76.5) years were retrospectively included in this analysis. All patients were consolidated with an allogeneic HSCT at the University Medical Center Leipzig between August 2007 and December 2020 in first (54%) or second (9%) CR or CR with incomplete peripheral recovery (18%) or with active disease (19%). Median follow up after allogeneic HSCT for patients alive was 3 years. Written informed consent was obtained from all patients in accordance with the Declaration of Helsinki.

Prior to allogeneic HSCT, patients received age-dependent induction chemotherapy protocols: of AML patients younger than 60 years at diagnosis (n=142, 48%), 111 patients received chemotherapy according to the AML 2002 study (OSHO #061),[90] six patients received chemotherapy within the RATIFY trial,[91] four patients were treated within the Quantum first trial (ClinicalTrials.gov Identifier: NCT02668653), four patients were treated within the PKC wild-type trial (ClinicalTrials.gov Identifier: NCT03512197), seven patients received “7+3” alone, five patients received CPX-351, five patients received sequential 5-azacitidine and chemotherapy, and one patient was diagnosed with AML as a child and was treated within the AML BFM-2004 study.[92] Among AML patients older than 60 years at diagnosis (n=152, 52%), 86 patients were treated within the AML 2004 study (OSHO #069),[93] 36 patients were treated within the OSHO #083 protocol, 14 patients received “7+3” alone, six patients received CPX-351, four patients were treated within the PKC wild-type trial (ClinicalTrials.gov Identifier: NCT03512197), three patients were treated within the Quantum first trial (ClinicalTrials.gov Identifier: NCT02668653), and one patient received upfront allogeneic HSCT.

5.1.2. Allogeneic HSCT and immunosuppression

The majority of patients (n=151; 53%) received NMA-HSCT with 3x30 mg/m^2 fludarabine and 2 Gy (n=155) or 3 Gy (n=4) TBI.[94] 70 patients (24%) received RIC-HSCT, either with combinations of fludarabine, cytarabine, and amsacrine (FLAMSA, n=37),[95] or with 5x30 mg/m^2 fludarabine combined with either 140 mg/m^2 melphalan (n=10),[96] busulfan (8mg/kg orally or 6.4 mg/kg intravenously, n=20), or 3x10 g/m^2 treosulfan (n=3).[97] Sixty-nine patients (23%) received MAC-HSCT with either cyclophosphamide 60 mg/kg body weight for two days and 12 Gy TBI (n=50), or 4x30 mg/m^2 fludarabine combined with 8 Gy TBI (n=19).

For prevention of GvHD, all patients received an intravenous starting dose of 5 mg/kg body weight cyclosporine A in two daily doses from day -1 which was adjusted to a whole-blood target level of 120-150 ng/ml for patients receiving FLAMSA conditioning or 200 ng/ml for all others. Cyclosporine A was tapered from day +42 and stopped on day 120 after FLAMSA conditioning and for all others tapered from day +84 or day +180 following related or unrelated HSCT, respectively.

Additionally, patients undergoing NMA-HSCT received mycophenolate mofetil 3 g per day in three daily doses after HSCT from an unrelated donor or 2 g per day in two daily doses after HSCT from a related donor. Patients receiving FLAMSA conditioning also received 2 g mycophenolate mofetil per day in two daily doses, which was stopped at day 28. Patients undergoing RIC- or MAC-HSCT additionally received 15 mg i.v. methotrexate on days +1, +3, +6, and +11 after HSCT, and *in vivo* T-cell depletion with thymoglobulin 2 mg/kg per day for three days when transplanted from an unrelated donor. After NMA-HSCT from a related donor, mycophenolate mofetil was stopped at day +28 or tapered from day +40 to +96 after unrelated HSCT.[94]

5.1.3. Assessment of GvHD

The incidence of acute and chronic GvHD was evaluated adapting the Glucksberg grading system.[98] Immunosuppression was prolonged or extended with systemic steroids in cases of GvHD (grade > 2

according to Glucksberg grading system).[98] Requirement for acute GvHD was engraftment while requirement for chronic GvHD was engraftment and survival for at least 100 days after HSCT.

5.2. Disease characterization

5.2.1. Evaluation at AML diagnosis

5.2.1.1. Morphology

At AML diagnosis, the percentages of blood and marrow blasts were evaluated using light microscopy. For analysis of peripheral blood, at least 200 cells were counted while for the analysis of bone marrow, at least 500 cells were counted. Hemoglobin levels and platelet counts were assessed using the full-automated blood cell counter, Sysmex XN-530 (Sysmex Corporation Ltd., Kobe, Japan).

5.2.1.2. Flow cytometry

At diagnosis, EDTA-anticoagulated bone marrow mononuclear cells were measured for their surface antigen expression of an institutional standard myeloid panel as described by Jentzsch *et al.*[19,99] Briefly, 100µl bone marrow blood were incubated for 15 minutes with labeled monoclonal antibodies. Afterwards, erythrocytes were lysed, samples washed in 1ml phosphate buffered saline solution (PBS) acid, fixed in 500µl PBS acid with 1% formaldehyde and 10,000 cells per sample were analyzed on the FACSCalibur flow cytometer adapting the CELLQUEST software (Becton Dickinson).

5.2.1.3. Genetic analyses

Cytogenetic analyses at diagnosis were performed using standard techniques of banding and *in situ* hybridization. The mutation status of the genes *CEBPA* and *NPM1* as well as the presence or absence of *FLT3*-ITD were evaluated as described by Bill *et al.*[42] Additionally, in 68 patients genomic DNA at diagnosis was available to perform targeted amplicon sequencing of 54 genes recurrently mutated in myeloid malignancies and included in the TruSight Myeloid Sequencing Panel (Illumina, San Diego, CA, USA) on the MiSeq platform (Illumina, San Diego, CA, USA) as described by Grimm *et al.*[100] Canonical

ASXL1 mutations at codon 646 were validated by Sanger sequencing approach with a proof-reading polymerase.[100] Patients were grouped into three risk groups according to the ELN2017 recommendations.[47]

5.2.1.4. Evaluation of RDW levels

The RDW was derived from the red blood cell distribution curves generated by an automated hematology analyzer Sysmex XN-530 (Sysmex Corporation Ltd., Kobe, Japan).

5.2.2. Evaluation at HSCT

5.2.2.1. Definition of remission status at HSCT

Prior to allogeneic HSCT, the remission status was assessed up to 28 days before the start of conditioning regimen in blood and bone marrow. CR was defined according to the ELN2010 criteria as normalization of bone marrow (*i.e.* <5%) and peripheral blood (*i.e.* 0%) blast counts, absence of blasts with Auer rods, with peripheral blood count regeneration (*i.e.* neutrophil count >1.0 Gpt/L, platelets >100 Gpt/L, and independence of blood transfusion) and no evidence of extramedullary disease.[101] For presence of CRi, all criteria for CR had to be met with the exception of platelets (<100 Gpt/L) or neutrophil count (<1.0 Gpt/L). In patients receiving allogeneic HSCT, the presence of CR or CRi was confirmed within 28 days prior to allogeneic HSCT by bone marrow and peripheral blood analysis. Active disease at allogeneic HSCT was defined by a persisting blast count >5% in bone marrow, persisting blasts in peripheral blood or the detection of extramedullary disease.

5.2.2.2. Evaluation of measurable residual disease at HSCT

Of the patients transplanted without evidence of active disease, peripheral blood or bone marrow up to 28 days prior to the start of conditioning regimen were available for MRD analysis in 150 patients. MRD status was assessed using digital droplet PCR for up to four previously published MRD markers.[102–105] In patients with a *NPM1* mutation detected at diagnosis, *NPM1* mutation-based MRD was evaluated

adapting a mutation-specific digital droplet PCR assay and MRD was defined as positive when *NPM1* was detectable at any level, as described by Bill *et al*.[105] Additionally, independent from the mutational profile at diagnosis, MRD based on expression levels of the AML-associated genes *BAALC/ABL1, MN1/ABL1* and *WT1/ABL1* was evaluated by digital droplet PCR or quantitative reverse transcriptase PCR adapting the previously published cut-offs.[102–104] For further analyses, patients with at least one positive MRD test were regarded as MRD positive at HSCT.

5.3. Statistical Analyses

5.3.1. Associations

All statistical analyses were performed using the R statistical software platform (version 3.4.3).[106] Associations with baseline clinical and genetic parameters were compared using the Kruskal-Wallis-Test for continuous parameters or the Fisher's exact tests for categorical variables.

5.3.2. Clinical endpoints

OS and EFS were calculated from allogeneic HSCT until death from any cause and relapse or death from any cause, respectively. For univariate analyses, survival estimates were calculated using the Kaplan-Meier method and groups were compared using the log-rank test. The competing risks NRM and cumulative incidence of relapse (CIR) were calculated from allogeneic HSCT to relapse/progression or death, respectively, adapting the Fine and Gray model.[107]

5.3.3. Definition of an optimal cut-point for RDW levels

With the R package "OptimalCutpoints" a receiver operator characteristic (ROC) based analysis was performed to identify a 20.7% cut-off to differentiate between patients dying from any cause during follow up after HSCT and patients staying alive.[108] This 20.7% cut-off was adapted to dichotomize the cohort in patients with high or low RDW levels at diagnosis.

5.3.4. Multivariate analyses

For the endpoints with significant outcome differences in univariate analyses (*i.e.* NRM and OS), multivariate proportional hazard models were constructed to evaluate the prognostic impact of RDW levels at diagnosis by backward adjusting for other relevant variables in AML.

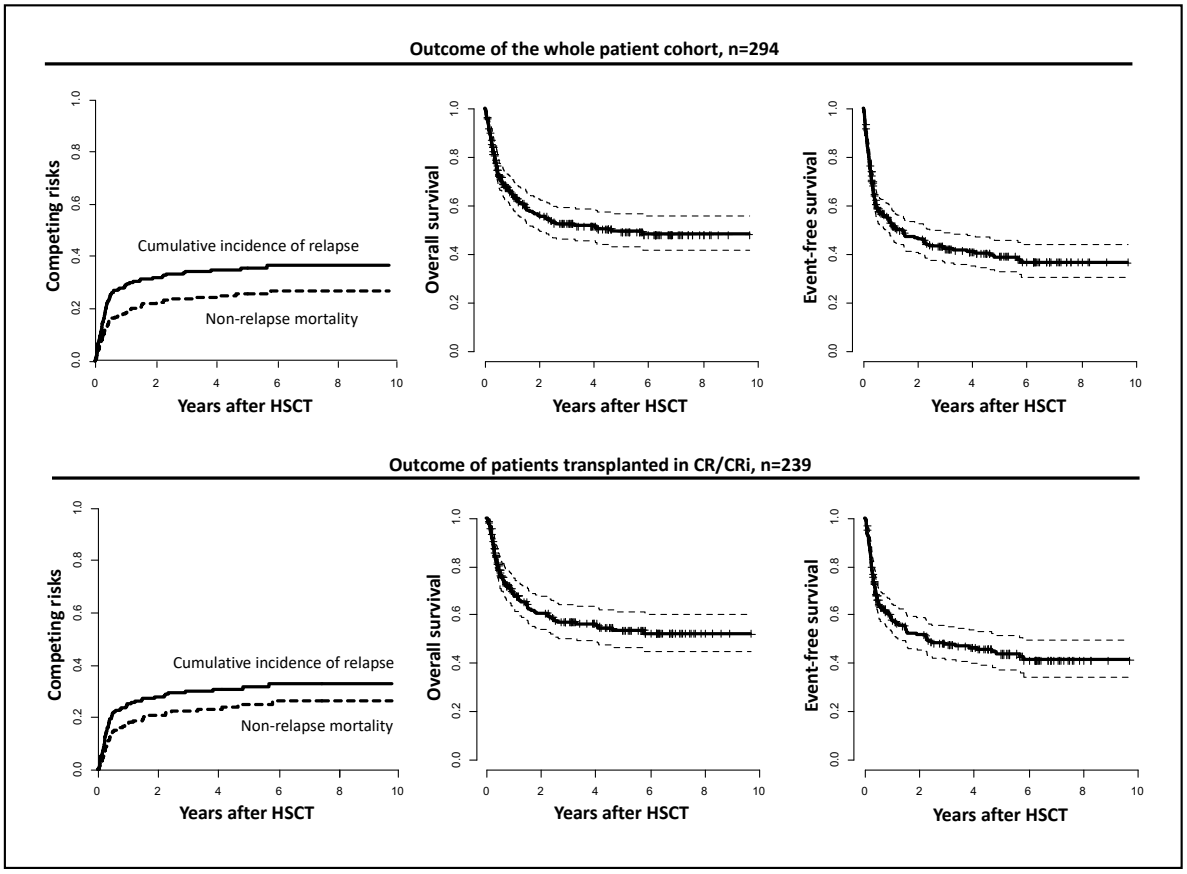
The following variables were considered for multivariable analyses: sex, age at diagnosis, disease origin (*de novo vs* secondary), ECOG score at diagnosis, the BMI at diagnosis (>35 *vs* 30-34.9 *vs* 25-29.9 *vs* 18.5-24.9 *vs* <18.8), ELN2017 genetic risk, disease status at HSCT (MRD^{neg} *vs* MRD^{pos} *vs* active disease), the HCT-CI risk score at HSCT (3 and more *vs* 1/2 *vs* 0 points), cytomegalovirus (CMV) status of recipient and donor (high-risk [+/-] *vs* all others), donor type (matched related *vs* matched unrelated *vs* mismatched unrelated), and sex of the donor (female into male *vs* all others). Of these, variables significant at α=.10 in univariate analyses were considered for the multivariate models. For both endpoints, hazard/odds ratios with their 95% confidence intervals are indicated for every significant prognostic factor included in the final model.

6. Ergebnisse / Results

6.1. Overall outcomes of the patient cohort

For the whole patient cohort (n=294), at three and five years after HSCT, CIR was 34% (Confidence interval [CI] 28%-40%) and 36% (CI 30%-42%), respectively, NRM was 24% (CI 19%-29%) and 26% (20%-31%), respectively, OS was 53% (CI 47%-59%) and 50% (CI 43-57%), respectively, and EFS was 42% (range 37%-49%) and 39% (CI 33%-46%), respectively. As expected, there were better outcomes observed for patients with a morphologic remission at the time of HSCT (n=239). Here, at three and five years after HSCT, CIR was 30% (CI 24%-36%) and 31% (CI 25%-38%), respectively, NRM was 22% (CI 17%-28%) and 25% (19%-31%), respectively, OS was 57% (CI 51%-64%) and 54% (CI 47%-62%), respectively, and EFS was 48% (range 41%-55%) and 44% (CI 37%-51%), respectively. Figure 3 shows overall outcomes for both patient populations.

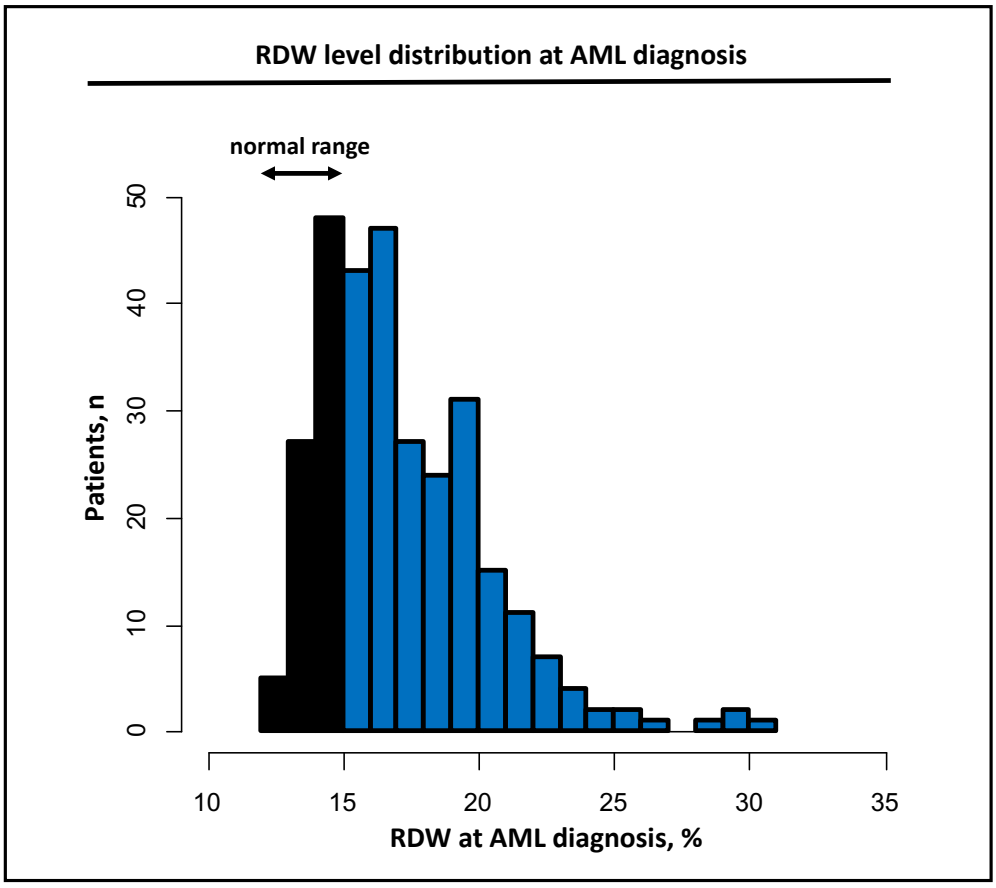
Figure 3. Overall outcomes for the whole patient cohort (n=294) and patients transplanted in morphologic remission (n=239).



6.2. RDW levels at AML diagnosis regarded as continuous parameter

The RDW levels at AML diagnosis before the start of cytoreductive therapies were highly variable (median 16.6%, range 12%-30.6%) and above the upper limit of normal (>15%) in 73% of analyzed patients (n=216, Figure 4).

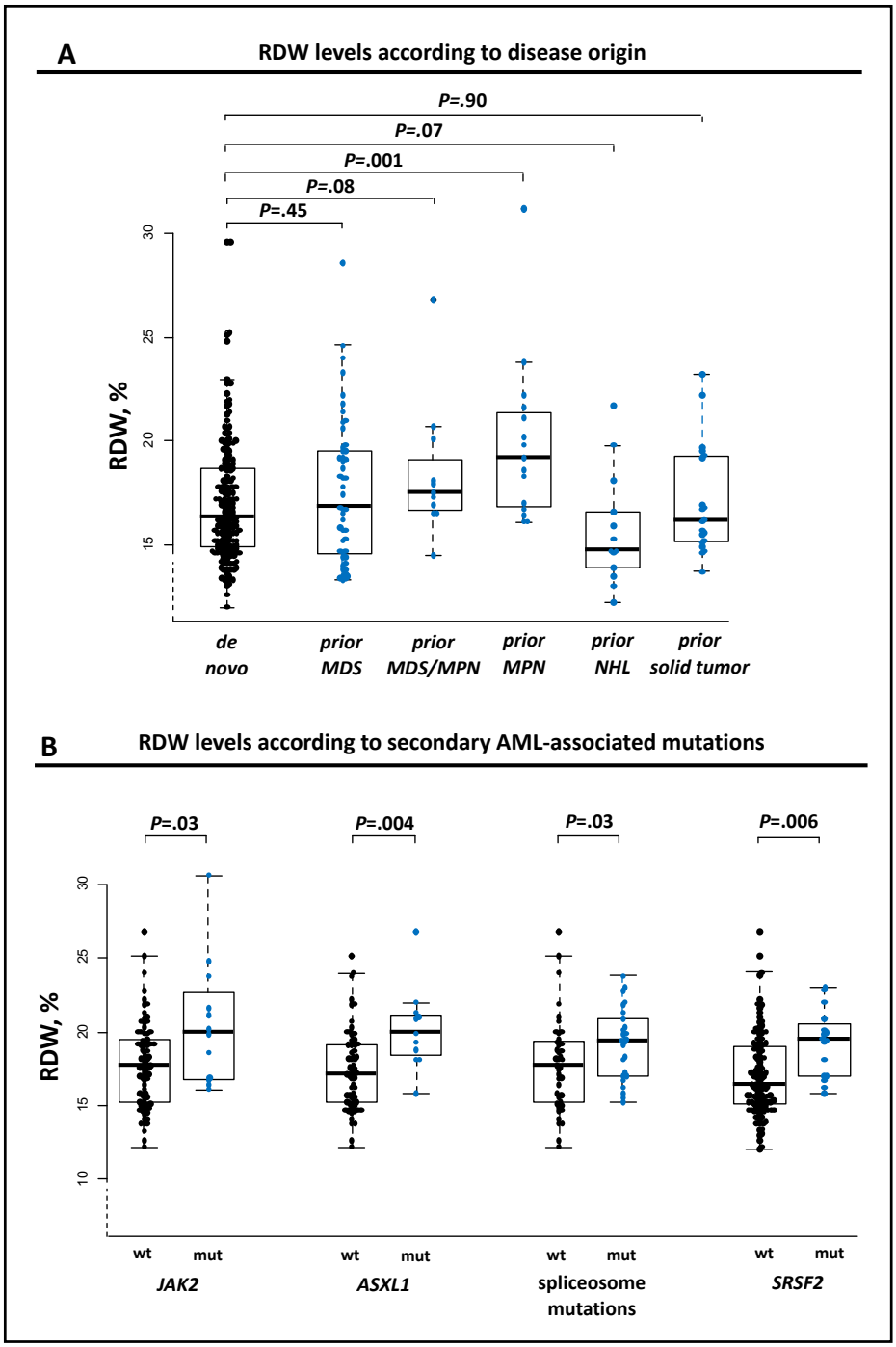
Figure 4. Distribution of RDW levels at diagnosis of AML in the whole patient cohort (n=294).



RDW levels of AML patients with a history of MDS or a solid tumor did not differ significantly from that of patients with *de novo* AML (*P*=.45 and *P*=.90, respectively). In contrast, AML patients with a history of myelodysplastic/myeloproliferative neoplasm (MDS/MPN) had a trend for higher RDW levels (*P*=.08), patients with a history of MPN significantly higher RDW levels (*P*=.001), and patients with a history of non-hodgkin lymphoma (NHL) a trend for lower RDW levels (*P*=.07, Figure 5A). Additionally, we also observed higher RDW levels in patients harboring gene mutations that have been previously linked to AML of secondary origin.[109,110] Compared to patients with a wild-type mutational status, RDW

levels were significantly higher in patients with a *JAK2* mutation (*P*=.03), an *ASXL1* mutation (*P*=.004), or a spliceosome mutation (*P*=.03, compromising *SF3B1*, *SRSF2*, *U2AF1*, and *ZRSR2,* Figure 5B), which was particularly driven by *SRSF2* mutations (*P*=.002).

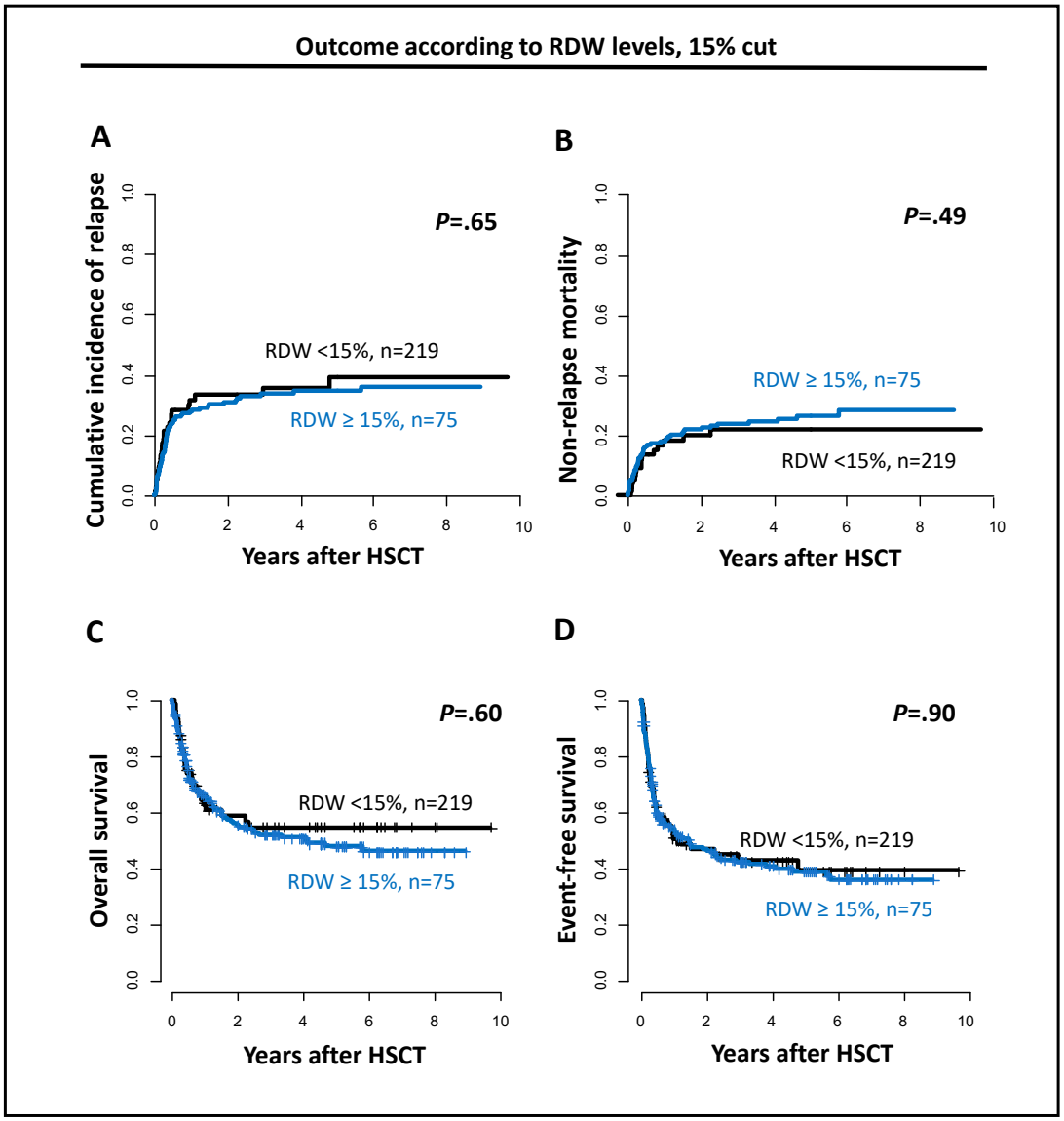
Figure 5. Associations of RDW levels at diagnosis (analyzed as continuous parameter) in the whole patient cohort (n=294).



6.3. The role of RDW levels at diagnosis as a predictor for outcomes after allogeneic HSCT

Adapting an upper limit of normal cut (15%) of RDW levels, there were no significant outcome differences between patients with high or low RDW levels at diagnosis regarding all analyzed endpoints, *i.e.* CIR (*P*=.65), NRM (*P*=.49), OS (*P*=.60), and EFS (*P*=.90, Figure 6).

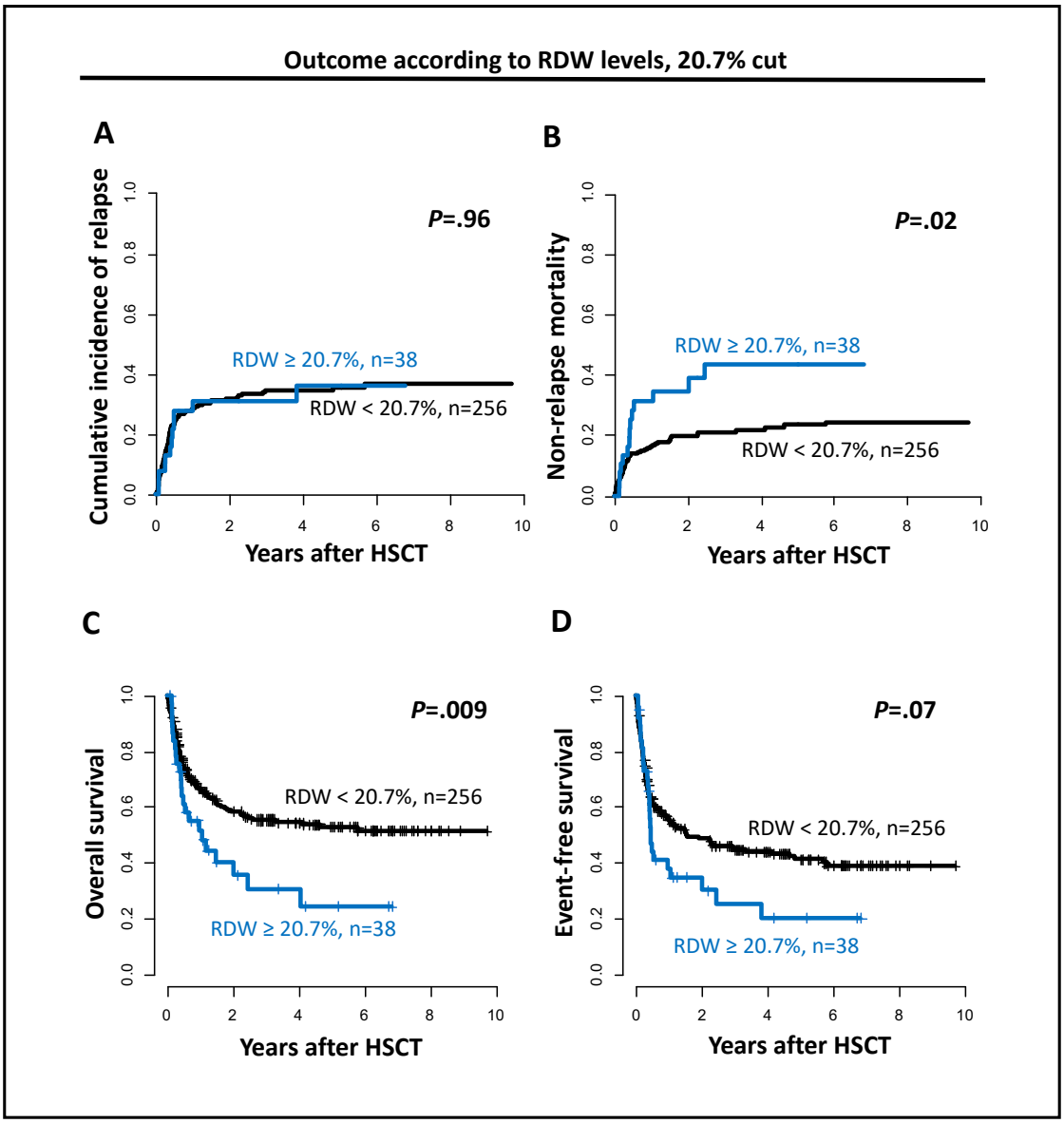
Figure 6. Outcome of the whole patient cohort according to RDW levels at diagnosis adapting an upper limit of normal cut (high *vs* low, 15% cut, n=294).



For further analyses, the defined optimal cut-point of 20.7% for RDW levels to dichotomize AML patients with high (n=38, 13%) and low (n=256, 87%) RDW levels at diagnosis. Adapting this cut-point,

patients with high RDW levels at diagnosis had a significantly higher NRM (*P*=.02) which also translated into significantly shorter OS (*P*=.009) and a trend for shorter EFS (*P*=.07), while CIR did not differ between both groups (*P*=.96, Figure 7).

Figure 7. Outcome of the whole patient cohort according to RDW levels at diagnosis adapting an optimal cut (high *vs* low, 20.7% cut, n=294).



The observed prognostic significance of RDW levels at diagnosis were tested for their independence from other clinically relevant parameters in AML in multivariate analyses for both significant

endpoints. Here, high RDW levels at diagnosis retained their association with higher NRM after adjustment for the BMI value at diagnosis (Table 3). Compared to patients with low RDW levels at diagnosis, patients with high RDW levels at diagnosis had a nearly doubled risk to die after HSCT without disease progression. In contrast, the only significant factors for OS in multivariate analysis were age at diagnosis, ELN2017 genetic risk and remission status at allogeneic HSCT.

Table 3. Multivariate analyses for the whole patient cohort.

	Cumulative incidence of non-relapse mortality		**Overall survival**	
	HR* (95% CI)	***P***	**OR** (95% CI)**	***P***
Age at Diagnosis, years (≥60 *vs* <60 years)	-	-	0.45 (0.28-0.72)	<.001
ELN2017 genetic risk (adverse *vs* intermediate *vs* favorable)	-	-	0.74 (0.56-0.99)	.04
RDW at diagnosis, % (high *vs* low, 20.7 cut)	1.86 (1.03-3.35)	.04	-	-
BMI at diagnosis, kg/m^2 (>35 *vs* 30-34.9 *vs* 25-29.9 *vs* 18.5-24.9 *vs* <18.8)	1.44 (1.11-1.87)	.006	-	-
Remission status at HSCT (active disease *vs* CR^{MRD+} *vs* CR^{MRD-})	-	-	0.66 (0.49-0.88)	.005

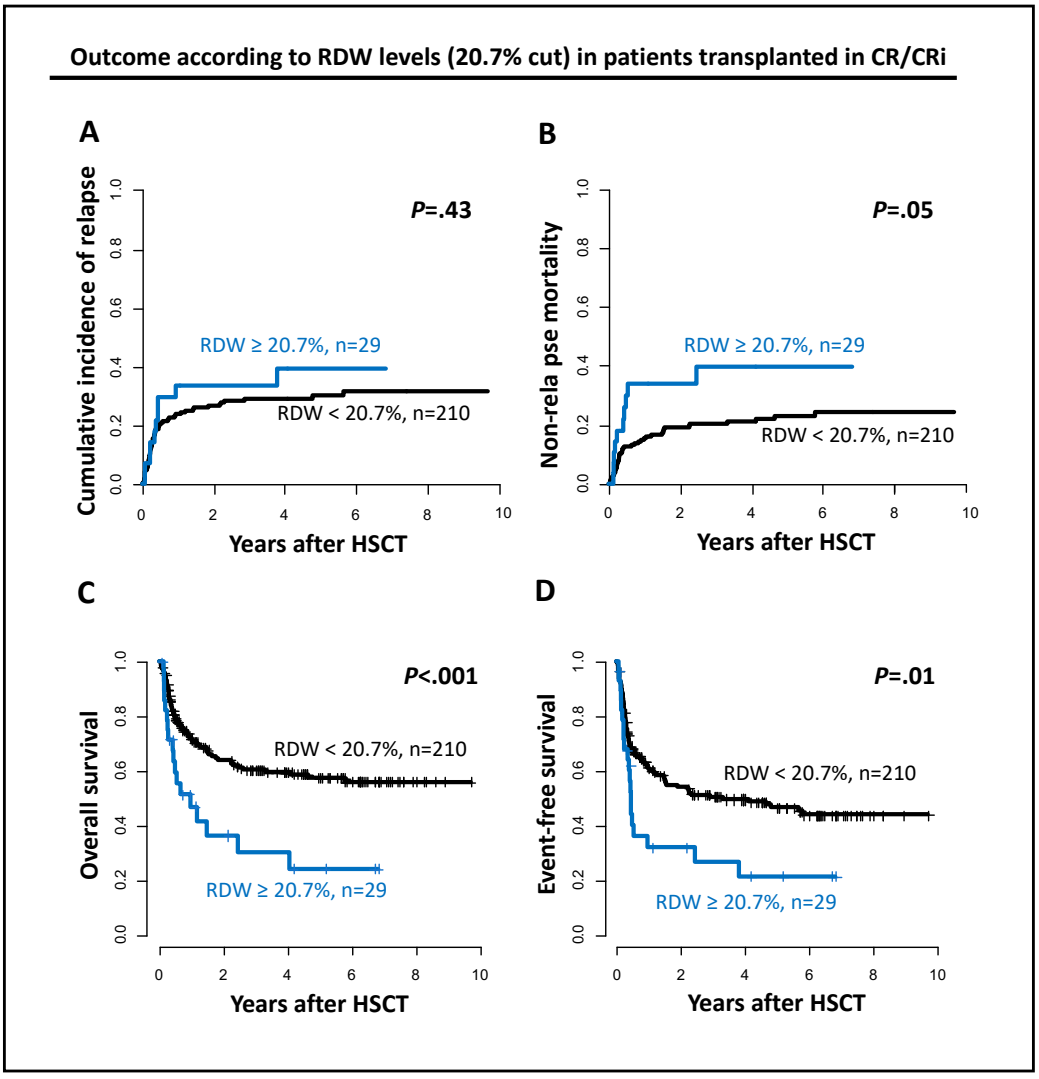
Abbreviations: CI, confidence interval; CR, complete remission; ELN, European LeukemiaNet; HSCT, hematopoietic stem cell transplantation; MRD, measurable residual disease.

*HR, hazard ratio, <1 (>1) indicates lower (higher) risk of relapse for the first category listed for the dichotomous variables.
**OR, odds ratio, <1 (>1) indicates lower (higher) chance of survival for the first category listed for the dichotomous variables.

Variables considered in the models were those significant at α=0.10 in univariable analyses.
For NRM endpoint, variables considered were age at diagnosis (≥60 ***vs*** < 60 years), RDW at diagnosis (high *vs* low, 20.7 cut), and BMI at diagnosis (>35 *vs* 30-34.9 *vs* 25-29.9 *vs* 18.5-24.9 *vs* <18.8).
For OS endpoint, variables considered were age at diagnosis (≥60 ***vs*** < 60 years), ELN2017 genetic risk group, disease origin (*de novo vs* secondary), RDW at diagnosis (high *vs* low, 20.7 cut), BMI at diagnosis (>35 *vs* 30-34.9 *vs* 25-29.9 *vs* 18.5-24.9 *vs* <18.8), the HCT-CI risk score at HSCT (3 and more *vs* 1/2 *vs* 0 points), and remission status at HSCT (active disease *vs* MRD^{pos} *vs* MRD^{neg}).

Similar results as in the whole patient cohort were derived when we restricted our analysis to AML patients transplanted in morphologic remission of their disease (n=239). Here, higher RDW levels associated with a significantly higher NRM (*P*=.05) as well as shorter OS (*P*<.001) and shorter EFS (*P*=.01) while CIR was not significantly different between both groups (*P*=.43, Figure 8).

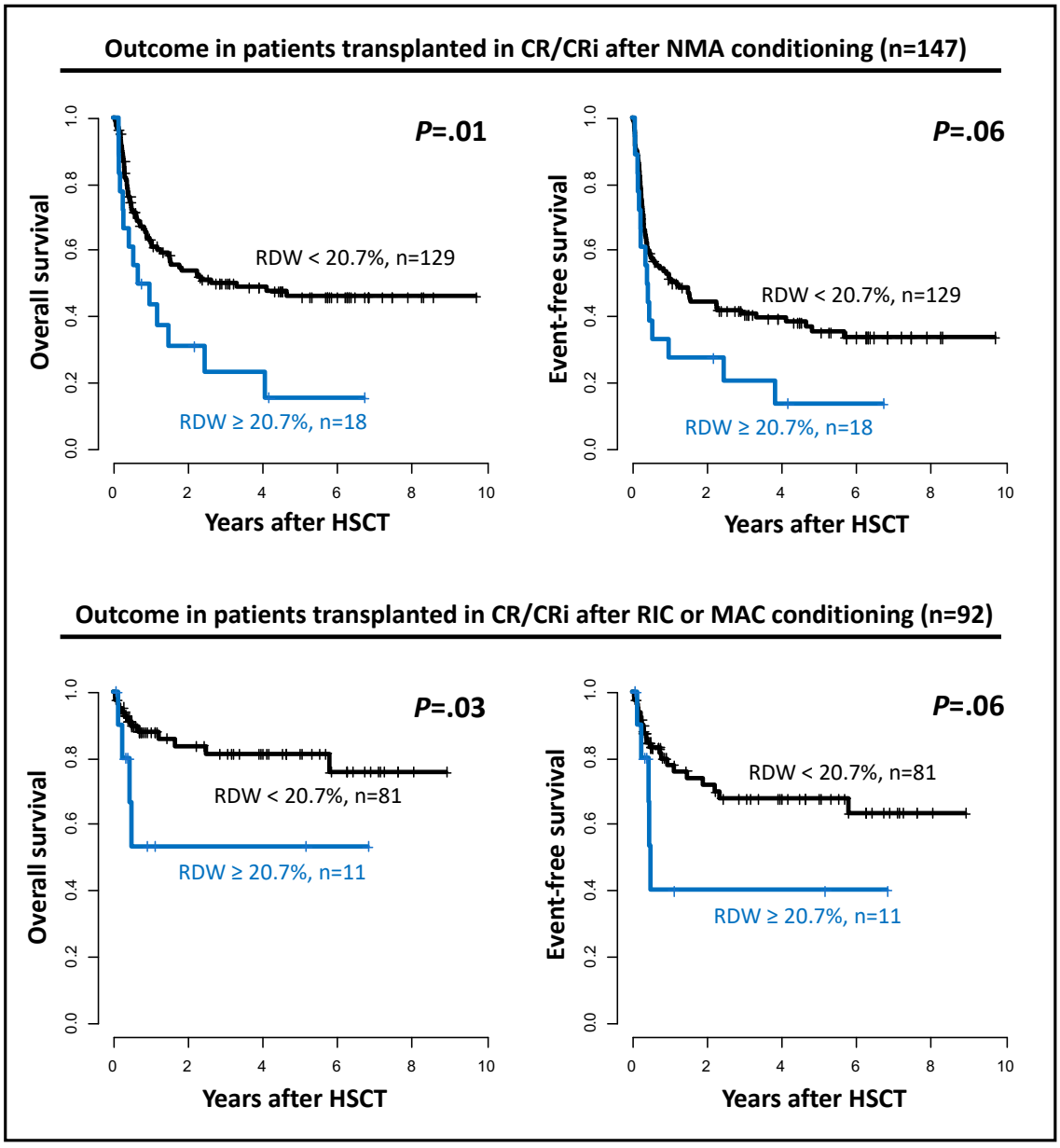
Figure 8. Outcome of patients transplanted in morphologic remission (n=239) according to RDW levels at diagnosis adapting an optimal cut (high *vs* low, 20.7% cut).



Finally, analyses were restricted to patients transplanted after NMA or patients transplanted after RIC/MAC conditioning (Figure 9). Also in these cohorts, a high RDW associated with shorter OS (*P*=.01

and *P*=.03, respectively) as well as a trend for shorter EFS (P=.06 and *P*=.06, respectively). These results indicate that the prognostic relevance of RDW levels at diagnosis remain independent from the applied conditioning intensity.

Figure 9. Outcome in AML patients transplanted in morphologic remission (n=239) according to RDW levels at diagnosis adapting an optimal cut (high *vs* low, 20.7% cut) in separate analysis according to the used conditioning regimen.



6.4. Associations of RDW levels at diagnosis

To shed light onto the characterization of patients with high or low RDW levels at diagnosis adapting the optimal cut-point, both groups were compared regarding their clinical, and genetic, and HSCT-related characteristics.

Patients with high RDW levels at diagnosis were more likely to harbor a secondary AML (*P*=.05) and had a lower hemoglobin level (*P*=.002), as well as lower bone marrow and blood percentages at diagnosis (*P*=.007 and *P*=.03, respectively). They also had a trend for a lower incidence of *NPM1* mutations (*P*=.06), but a higher incidence of *ASXL1* (*P*=.02), *JAK2* (*P*=.05), and by trend *SRSF2* mutations (*P*=.09). In contrast, all analyzed HSCT-related characteristics, including donor type, conditioning regimens, and the comorbidity score HCT-CI did not differ significantly between both patient groups.

We also observed differences in the immunophenotype of patients with high or low RDW levels at diagnosis. While the burden of the CD34+/CD38- cell population – which is known to harbor the majority of LSCs – did not differ between both patient populations, patients with high RDW levels had a significantly lower expression of surface antigens indicating myeloid (CD13, *P*<.001 and CD33, *P*=.002) or monocyte differentiation (CD64, *P*=.08), a lower expression of the pan-leukocyte antigens CD38 (*P*=.001) and CD45 (*P*=.002) and a lower expression of the immature antigen CD117 (*P*=.02). In contrast, there was a higher expression indicating erythrocyte (Glykophorin A, *P*=.02) and thrombocyte differentiation (CD61, *P*=.06). Table 4 displays all analyzed characteristics and their distribution between patients with high or low RDW levels at diagnosis.

Table 4. Clinical, genetic, and HSCT-related characteristics in AML patients consolidated with an allogeneic HSCT according to RDW levels at diagnosis (high *vs* low, 20.7% cut, n=294).

Characteristics	**All patients, n=294**	**low RDW, n=256**	**high RDW, n=38**	***P***
Clinical parameters at diagnosis				
Age at diagnosis, years				.73
< 60 years	142	125 (49)	17 (45)	
≥ 60 years	152	131 (51)	21 (55)	
Sex, n (%)				.12
male	158	133 (52)	25 (66)	
female	136	123 (48)	13 (34)	
ECOG score at diagnosis, n (%)				.59
0	12	10 (10)	2 (17)	
1	50	46 (46)	4 (33)	
2	33	28 (28)	5 (42)	
3	16	15 (15)	1 (8)	
BMI at diagnosis, kg/m^2				.70
< 18.5	8	8 (3)	0 (0)	
18.5-24.9	106	93 (38)	13 (37)	
25-29.9	110	98 (40)	12 (34)	
30-34.9	45	37 (15)	8 (23)	
≥ 35	15	13 (5)	2 (6)	
Disease origin, n (%)				.05
de novo	184	166 (65)	18 (47)	
secondary	110	90 (35)	20 (53)	
Hemoglobin, g/dL				.002
median	8.4	8.9	7.9	
range	3.2-15.3	3.2-15.3	5.5-10.9	
Platelet count, x 10^9/L				.79
median	63	63	65	
range	1.6-950	3-488	2-950	
WBC count, x 10^9/L				.61
median	5.4	5.3	6.1	
range	0.1-366	0.1-366	0.6-325	
Percentage of blood blasts, %				.03
median	17	20	10	
range	0-97	0-97	0-93	
Percentage of BM blasts, %				.007
median	50	50	32	
range	0-95	0-95	11-90	
Genetic parameters at diagnosis				
Karyotype, n (%)				.72
abnormal	169	146 (58)	23 (62)	
normal	118	104 (42)	14 (38)	
ELN2017 Genetic Group, n (%)				.55
favorable	62	57 (25)	5 (16)	
intermediate	91	79 (34)	12 (38)	
adverse	109	94 (41)	15 (47)	
***NPM1*, n (%)**				.06

wild-type	210	179 (73)	31 (89)	
mutated	69	65 (27)	4 (11)	
***FLT3*-ITD, n (%)**				.11
absent	225	193 (79)	32 (91)	
present	54	51 (21)	3 (9)	
***CEBPA*, n (%)**				.35
wild-type	218	192 (90)	26 (84)	
mutated	26	21 (10)	5 (16)	
***DNMT3A,* n (%)**				.51
wild-type	69	57 (72)	12 (86)	
mutated	24	22 (28)	2 (14)	
***FLT3*-TKD, n (%)**				.33
wild-type	252	218 (91)	34 (97)	
mutated	22	21 (9)	1 (3)	
***RUNX1,* n (%)**				.68
wild-type	57	47 (82)	10 (91)	
mutated	11	10 (18)	1 (9)	
***TP53,* n (%)**				.39
wild-type	58	50 (85)	8 (73)	
mutated	12	9 (15)	3 (23)	
***ASXL1,* n (%)**				.02
wild-type	60	53 (90)	7 (58)	
mutated	11	6 (10)	5 (42)	
***IDH1,* n (%)**				.70
wild-type	127	107 (88)	20 (95)	
mutated	15	14 (12)	1 (5)	
***IDH2,* n (%)**				.48
wild-type	126	109 (88)	17 (81)	
mutated	19	15 (12)	4 (19)	
***JAK2,* n (%)**				.05
wild-type	77	65 (90)	12 (71)	
mutated	11	7 (10)	5 (29)	
***SRSF2,* n (%)**				.09
wild-type	113	102 (89)	11 (73)	
mutated	16	12 (11)	4 (27)	
Spliceosome mutation,* n (%)				.21
absent	41	35 (66)	6 (46)	
present	25	18 (34)	7 (54)	
Flow cytometry at diagnosis				
Bone marrow CD2 expression, %				.18
median	14	14	20.5	
range	1.5-81	1.5-81	2-78	
Bone marrow CD7 expression, %				.17
median	17	16	24.5	
range	2-96	2-96	2-86	
Bone marrow CD11b expression, %				.83
median	13.5	14	12.5	
range		0.5-92	2-78	
Bone marrow CD13 expression, %				<.001
median	55	57	32	
range	0.5-97	0.5-97	5-75	
Bone marrow CD14 expression, %				.20
median	3	2	3.5	
range	0.5-70	0.5-70	0.5-52	
Bone marrow CD15 expression, %				.11

median	26	27	21.5	
range	1-97	1-97	3-94	
Bone marrow CD33 expression, %				.002
median	62	64	41	
range	1-98	1-98	2-92	
Bone marrow CD34 expression, %				.37
median	22	25.6	18.3	
range	0-97	0-97	0.2-81.5	
Bone marrow CD38 expression, %				.001
median	71.5	73	56.5	
range	4-98	4-98	15-92	
BM CD34+/CD38- burden, %				.48
median	0.8	1	0.7	
range	0-89	0-89	0-39.5	
Bone marrow CD45 expression, %				.002
median	91	92.5	82	
range	12-100	12-100	23-99	
Bone marrow CD56 expression, %				.32
median	9	8	11	
range	0.5-94	0.5-94	2-78	
Bone marrow CD61 expression, %				.06
median	4	4	8	
range	0.5-56	0.5-56	0.5-48	
Bone marrow CD64 expression, %				.08
median	15	17	8.5	
range	0-95	0-95	1-89	
Bone marrow CD65 expression, %				.30
median	18	19	13	
range	0.5-92	0.5-92	3-91	
Bone marrow CD117 expression, %				.02
median	34	35.5	22	
range	0.5-96	0.5-96	2-79	
Bone marrow GlyA expression, %				.02
median	11	11	15	
range	0.5-90	0.5-90	1-72	
HSCT-related parameters				
Remission status at HSCT, n (%)				.23
CR^{MRD-}	89	78 (43)	11 (46)	
CR^{MRD+}	61	57 (32)	4 (17)	
active disease	54	45 (25)	9 (38)	
HCT-CI score				.77
0	97	85 (36)	12 (32)	
1/2	81	68 (29)	13 (35)	
≥3	93	81 (35)	12 (32)	
Conditioning regimen, n (%)				.92
MAC	69	61 (24)	8 (21)	
RIC	70	60 (23)	10 (26)	
NMA	155	135 (53)	20 (53)	
Donor type, n (%)				.94
related	47	41 (16)	6 (16)	
unrelated, HLA matched	188	164 (64)	24 (63)	
HLA mismatched	46	39 (15)	7 (18)	
haploidentical	13	12 (5)	1 (3)	
Donor sex, n (%)				.63
female into male	46	39 (15)	7 (18)	

all others	248	217 (85)	31 (82)	
CMV status, n (%)				.72
recipient + / donor –	105	93 (36)	12 (32)	
all others	189	163 (64)	26 (68)	
acute GvHD >= grade 2, n (%)		169 (76)		1
absent	196	53 (24)	27 (77)	
present	61		8 (23)	
chronic GvHD, n (%)				.63
absent	97	88 (50)	9 (50)	
limited	30	26 (15)	4 (22)	
extensive	67	62 (35)	5 (28)	

Abbreviations: BM, bone marrow; BMI, body mass index; CD, cluster of differentiation; CEBPA, CCAAT/enhancer-binding protein alpha; CMV, cytomegalovirus; DNMT3A, DNA-methyltransferase 3A gene; ECOG, eastern cooperative oncology group; ELN, European LeukemiaNet; FLT3-ITD, internal tandem duplication of the FLT3 gene; FLT3-TKD, tyrosine kinase domain of the FLT3 gene; GlyA, Glykophorin A; GvHD, graft-versus-host disease; HCT-CI, Hematopoietic Cell Transplantation-specific Comorbidity Index; HLA, human leucocyte antigen; IDH1, isocitrat dehydrogenase 1; IDH2, isocitrat dehydrogenase 2; JAK2, janus kinase 2; MAC, myeloablative; MRD, measurable desidual disease; NMA, non-myeloablative; NPM1, nucleophosmin-1; RIC, reduced intensity conditioning; RUNX1, Runt-related transcription factor 1; SRSF2, Serine And Arginine Rich Splicing Factor 2; TP53, tumor protein 53; WBC, white blood cell.

* spliceosome mutations, compromising *SF3B1*, *SRSF2*, *U2AF1*, and *ZRSR2.*

7. Diskussion / Discussion

As the RDW is part of the output of most automated blood cell counter, it is included in the diagnostic work up of most hematologic diseases. Across their lifespan, red blood cells typically decrease in cellular volume which is why a delayed clearance of older erythrocytes leads to higher RDW levels.[71] Subsequently, a higher RDW mirrors dysregulated erythrocyte homeostasis with either impaired erythropoiesis or abnormal (prolonged) red blood cell survival. While historically, the clinical meaning of the RDW was limited to the differential diagnosis of anemia, a wider applicability became increasingly evident. An increase in RDW levels has been shown to associate with a generally increased mortality in the healthy population and linked to a variety of irregularities, including oxidative stress, poor nutritional status, but also older age.[73,74] Data also suggest that a high RDW defines a pro-inflammatory state,[75] and has been connected with several cardiovascular diseases – including atrial fibrillation, heart failure, coronary heart disease, and cardiac mortality as well as a higher likelihood to develop a variety of malignancies.[76]

A previous study showed that healthy individuals with detectable somatic mutations – which are known to confer a higher risk of developing a myeloid neoplasm – had higher RDW (>14.5%) than unmutated patients.[85] In individuals with unexplained cytopenias, a high RDW was an independent factor that predicted the diagnosis of MDS[89] and among the most important factors to discriminate healthy blood samples from that of MDS patients.[86] Additionally, in healthy individuals a higher RDW associates with a higher risk of developing AML, already several years before diagnosis.[76,89] However, no study assessed the clinical value of RDW levels in patients diagnosed with AML, or in the context of an allogeneic HSCT. Here, patient selection is of upmost importance to achieve long-term outcomes, as it is the consolidation option with the highest chance of cure but also harbors the risk of significant treatment-related mortality.

Regarding the prognostic relevance at diagnosis of a myeloid neoplasm, higher-than-normal RDW levels have been linked to worse event-free and treatment-free survival in chronic myeloid leukemia (CML),[111] and shorter OS in MDS patients[87,112] In this study – which is the first to analyze the prognostic

relevance in AML patients – there was no prognostic significance of a RDW above the upper limit of normal range (*i.e.* 15%). Patients with higher-than-normal RDW levels had similar CIR (*P*=.65), NRM (*P*=.49), OS (*P*=.60), and EFS (*P*=.90, Figure 6). However, when introducing an optimal cut of 20.7% derived by ROC statistics, higher RDW levels associated with a significantly higher NRM (*P*=.02), which also translated into shorter OS (*P*=.009) and a trend for shorter EFS (*P*=.07) but similar CIR (*P*=.96, Figure 7). In multivariate analyses, the RDW retained its' prognostic significance for a higher NRM after adjustment for the BMI value at diagnosis (Table 3). Similar results were obtained when the analysis was restricted to patients transplanted in morphologic remission (CIR, *P*=.43, NRM, *P*=.05, OS, *P*<.001, EFS, *P*=.02, Figure 8). In the according to the optimal cut-point dichotomized cohort, a high RWD associated with secondary AML (*P*=.05, Table 4), and mutations of genes associated with secondary AML origin, as *JAK2* (*P*=.05), *ASXL1* (*P*=.02), and by trend *SRSF2* (*P*=.09), and a trend for a lower incidence of *NPM1* mutations (*P*=.06) which usually occur in *de novo* AML. In MDS patients a high RDW was shown to associate with low hemoglobin levels and higher neutrophils,[112] and in CML patients with female sex, a higher white blood count, higher blast percentages, and lower hemoglobin levels.[111] Also in this AML cohort, a high RDW associated with lower hemoglobin levels (*P*=.002), but also lower blood and bone marrow blast percentages (*P*=.03 and *P*=.007, respectively). Similar to data in MDS,[87,112] RDW levels did not associate with chromosomal abnormalities or disease risk according to the currently usually adapted genetic risk stratification system (ELN2017). Despite the described association of a high RDW with a higher cardiovascular and inflammatory risk, in this study, neither the BMI at diagnosis (P=.70), nor the HCT-CI risk score at HSCT (*P*=.77) or the risk of developing an acute or chronic GvHD, (*P*=1 and *P*=.63, respectively) differed according to RDW levels in AML patients.

In conclusion, this study is the first to evaluate the RDW in newly diagnosed AML patients. Patients with a secondary AML or secondary-AML like gene mutations had higher RDW levels. While a secondary AML alone has been previously shown to not associate with survival in patients undergoing allogeneic HSCT when the individual genetic risk is considered,[37] the presence of secondary AML-like gene mutations seems to be able to identify patients with adverse outcomes.[109] This data point to the

fact that also the RDW – which is a cost-effective, universally available and fast clinical parameter - has the ability to identify AML patients with a high treatment-related mortality after allogeneic HSCT. This also seemed independent from the individual comorbidities (which are reflected in the HCT-CI score), donor selection, or the development of a GvHD– one of the most relevant risk factors for death after allogeneic HSCT. As allogeneic HSCT remains the consolidation therapy that provides the best disease control, this comes at the cost of a significant treatment-related mortality. Subsequently, carefully considered patient selection for this procedure remains of high importance, for which – after validation in prospective clinical studies - RDW evaluation may provide an important clinical value.

8. Zusammenfassung / Summary

Dissertation zur Erlangung des akademischen Grades Dr. med.

The Clinical Significance of Diagnostic Red Cell Distribution Width in Patients with Acute Myeloid Leukemia

eingereicht von Vladan Vucinic

angefertigt am

Universitätsklinikum Leipzig

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April 2021

Acute myeloid leukemia (AML) is a clinically and genetically a highly heterogeneous disease that results from the clonal expansion and impaired differentiation capability of myeloid blasts. An allogeneic hematopoietic stem cell transplantation (HSCT) is the consolidation option that offers the highest chance of relapse-free survival for AML patients, but is accompanied by a relevant treatment-related morbidity and mortality and thus, is offered to AML patients with a high relapse risk. Subsequently, individual risk stratification as well as accurate patient selection for intensive therapeutic approaches is of upmost importance.

The red blood cell distribution width (RDW) is a universally available clinical factor that in general increases with age, but also reflects dysregulated erythrocyte homeostasis. Historically, the RDW is an important factor in the differential diagnosis of anemia, but its' clinical significance beyond that

became increasingly evident. Today we know that a high RDW is a risk factor for morbidity and mortality in the apparently healthy population and also linked to a variety of diseases that accompany oxidative stress or inflammation. Subsequently, a high RDW has been linked to a higher incidence of cardiovascular diseases, including but not limited to coronary heart disease, heart failure and cardiac mortality. Also in the context of malignant diseases, the RDW has been mentioned to play a role in carcinogenesis, solid tumor progression as well as to identify high risk patients with a variety of hematologic disorders. Regarding myeloid neoplasm, a high RDW seems to be able to identify healthy individuals with an increased risk to develop a myelodysplastic syndrome (MDS) as well as AML, also several years before diagnosis. However, while data suggests a prognostic significance in patients with diagnosed MDS or chronic myeloid leukemia (CML), until today, no study evaluated the role of RDW levels in patients diagnosed with AML.

The first objective of the here presented study was to **assess the distribution of RDW levels in newly diagnosed AML patients**.

At AML diagnosis, RDW levels were highly variable with a median of 16.6% and ranged from 12% to 30.6%. Of all analyzed AML patients, 73% (n=216) showed values above the upper limit of normal.

The second objective of this study was to **evaluate the prognostic significance of RDW levels at diagnosis in AML patients consolidated with an allogeneic HSCT**.

When dichotomizing the patient cohort according to the upper level of normal cut (15%), no outcome differences between AML patients with a normal or an elevated RDW at diagnosis were observed. However, when introducing a receiver operator characteristic-derived optimal cut-point of 20.7%, distinct outcomes were observed. Patients with a high RDW (*i.e.* ≥ 20.7%, n=38) at diagnosis had similar cumulative incidence of relapse (CIR, *P*=.96), but a significantly higher non-relapse mortality (NRM, *P*=.02) which also translated into significantly shorter overall survival (OS, *P*=.009) and a trend for

shorter event-free survival (EFS, *P*=.07). Similar results were obtained when the analysis was restricted to patients transplanted without morphologic evidence of active disease in complete remission or complete remission with incomplete peripheral recovery. Here, NRM (*P*=.05), OS (*P*<.001), and EFS (*P*=.01) differed significantly between patients with high or low RDW levels at diagnosis.

The third objective of this study was to **evaluate whether RDW levels at AML diagnosis provide prognostic information independently from other relevant prognostic factors in AML**.

For this purpose, multivariate analyses were performed to assess the prognostic significance of RDW levels at diagnosis after backward selection for other prognostically relevant factors in AML. Here, a high RDW retained its' prognostic relevance after adjustment for the body mass index at diagnosis. Patients with high RDW levels had a nearly doubled risk to die without disease progression after HSCT (Hazard ratio 1.86, confidence interval 1.03-3.35, *P*=.04). Additionally, separate analyses for patients transplanted after non-myeloablative and patients transplanted after reduced intensity or myeloablative conditioning showed a significantly shorter OS (*P*=.01 and *P*=.03, respectively) and a trend for shorter EFS (*P*=.06 and *P*=.06, respectively). Subsequently, the prognostic value of RDW levels at diagnosis also seemed to be independent from the applied conditioning regimen.

The final objective of this study was to **evaluate associations of RDW levels at AML diagnosis with other clinically, genetically, and prognostically relevant factors in AML**.

Patients with RDW levels >20.7% at diagnosis were significantly more likely to harbor a secondary AML and had a lower hemoglobin level (*P*=.002), as well as lower bone marrow and blood percentages at diagnosis (*P*=.007 and *P*=.03, respectively). Additionally, a higher incidence of mutations known to associate with secondary AML, *i.e. ASXL1*, *JAK2*, and *SRSF2* mutations, but a trend for a lower incidence of *NPM1* mutations – which are enriched in *de novo* AML – were observed. In contrast, all analyzed patient-related factors, as the ECOG status at diagnosis or the comorbidity score HCT-CI at HSCT, as

well as HSCT-related characteristics, including donor type and conditioning regimens, did not differ significantly between both patient groups.

Taken together, the presented data indicate that the RDW at diagnosis of AML represents a cost-effective, universally available and fast clinical parameter – that has the ability to identify AML patients with a high treatment-related mortality after allogeneic HSCT. After validation in prospective clinical studies, evaluation of RDW at AML diagnosis may provide an important clinical value to select consolidation treatments in AML patients.

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10. Erklärung über die eigenständige Abfassung der Arbeit

Hiermit erkläre ich, dass ich die vorliegende Arbeit selbstständig und ohne unzulässige Hilfe oder Benutzung anderer als der angegebenen Hilfsmittel angefertigt habe. Ich versichere, dass Dritte von mir weder unmittelbar noch mittelbar eine Vergütung oder geldwerte Leistungen für Arbeiten erhalten haben, die im Zusammenhang mit dem Inhalt der vorgelegten Dissertation stehen, und dass die vorgelegte Arbeit weder im Inland noch im Ausland in gleicher oder ähnlicher Form einer anderen Prüfungsbehörde zum Zweck einer Promotion oder eines anderen Prüfungsverfahrens vorgelegt wurde. Alles aus anderen Quellen und von anderen Personen übernommene Material, das in der Arbeit verwendet wurde oder auf das direkt Bezug genommen wird, wurde als solches kenntlich gemacht. Insbesondere wurden alle Personen genannt, die direkt an der Entstehung der vorliegenden Arbeit beteiligt waren. Die aktuellen gesetzlichen Vorgaben in Bezug auf die Zulassung der klinischen Studien, die Bestimmungen des Tierschutzgesetzes, die Bestimmungen des Gentechnikgesetzes und die allgemeinen Datenschutzbestimmungen wurden eingehalten. Ich versichere, dass ich die Regelungen der Satzung der Universität Leipzig zur Sicherung guter wissenschaftlicher Praxis kenne und eingehalten habe.

Leipzig, 09.04.2021 Vladan Vucinic

11. Curriculum Vitae

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Ausbildung

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Beruflicher Werdegang

11/00 – 11/01	Arzt in Praktikum an Klinischem Zentrum Serbien, Belgrad
11/01 – 06/02	Arzt in Weiterbildung, Institut für Hämatologie, Klinisches Zentrum Serbien, Belgrad
07/02 – 04/05	Arzt in Weiterbildung, KMT Klinik Idar-Oberstein
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26.05.2004	Gleichwertigkeitsprüfung im Sinne der Approbationsordnung, Bezirksärztekammer Rhein-Hessen, Mainz

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18.12.2008	Facharztprüfung (Sächsische Landesärztekammer). Facharzt für Innere Medizin
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19.07.2011	Facharztprüfung (Sächsische Landesärztekammer). Facharzt für Innere Medizin, Hämatologie und internistische Onkologie
15.12.2011	Erteilung der deutschen Approbation
01.04.2012	Ernennung zum Oberarzt

Berufliche Weiterbildung

09.10.2010	Spezialkurs Röntgendiagnostik zum Erwerb der Fachkunde für Ärzte
07.09.2015	Erwerb der Bescheinigung über die erforderliche Fachkunde im Strahlenschutz
28.11.2015	Qualifikation zur fachgebundenen genetischen Beratung
14.03.2020	Moderator für Qualitätszirkel in der vertragsärztlichen/psychotherapeutischen Versorgung
02.12.2020	Hochschullehrtraining

Mitgliedschaften

Deutsche Gesellschaft für Innere Medizin

Deutsche Gesellschaft für Hämatologie und Onkologie

European Hematology Association

12. Komplette Publikationsliste (Peer-reviewed)

1. Linck, D., Basara, N., Tran, V., **Vucinic, V**., Hermann, S., Hoelzer, D., Fauser, A.A. Peracute onset of severe tumor lysis syndrome immediately after 4 Gy fractionated TBI as part of reduced intensity preparative regimen in a patient with T-ALL with high tumor burden. Bone Marrow Transplant. 2003 May;31(10):935-7.

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