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Validation of and proposals for refinements of the WHO 2016 classification for myelodysplastic syndromes

To the Editor:

Myelodysplastic syndromes (MDS) are generally introduced as bone marrow disorders that are notorious for their heterogeneity.[1–3] Then the question arises as to what is the best method for the disease classification of these heterogeneous syndromes? To improve the definition and rationale of the classification of MDS, the World Health Organisation (WHO) updated the 2008 classification recently.[4] As yet, these proposals have only been validated by Strupp et al.,[5] who considered the refined proposals of the refined WHO as being thoughtful. In this article, not only the clinical relevance of changes made in the WHO classification are explored, but also refinements are proposed to further improve the categorisation of these heterogeneous syndromes.

As compared to the 2008 edition, the WHO has made some important alterations in the latest classification. First, following this WHO-2016 proposal to reject the nonerythroid blast count rule, patients previously considered with acute erythroid leukaemia (AEL) are reclassified as MDS based on the absolute blast counts. Second, MDS patients with multilineage dysplasia and ring sideroblasts are again separated from patients lacking ring sideroblasts.[4] In the 2008 edition, these disease entities were merged together because of their prognostic similarity.[6,7] However, the recognised link between molecular abnormalities and morphology, i.e., SF3B1 mutations and ring sideroblasts, urges for separated disease entities, at least in MDS patients with lower than 5% bone marrow blasts.[8,9] Further acknowledging the shared pathophysiological pathway, the WHO 2016 guidelines allow for diagnosing ring sideroblasts at a level of $\geq$5% when accompanied by a SF3B1 mutation.[4]

A limited number of other adjustments has been made in the WHO 2016 classification, of which most are of semantic nature.[4] The name of MDS categories has been preceded by the preposition *refractory anaemia* since the introduction of the first morphological classification in 1982, although anaemia is not present in all MDS cases.[10,11] The WHO 2016 classification substituted the preposition *refractory anaemia* with the term *MDS*, resulting into disease categories as *MDS with* ring sideroblasts and *MDS with* excess blasts rather than *refractory anaemia* with ring sideroblasts and *refractory anaemia* with excess blasts.[4] Still, the WHO 2016 classification encompasses terms being potentially misleading. As in the WHO 2008 classification, the category MDS unclassifiable (MDS-U) is applicable only to patients fulfilling its straightforward criteria, e.g., pancytopaenia besides multilineage dysplasia (MDS-U Pan), and the presence of 1% peripheral blood blasts in cases with less than 5% bone marrow blasts (MDS-U PB).[4,12] In addition, based on the prognostic similarity of a single del(5q) abnormality and double cytogenetic abnormalities including del(5q), the category MDS with isolated del(5q) now includes patients with one additional chromosomal abnormality (with the exclusion of monosomy 7) as well.[4]

To validate the WHO 2016 classification, we conducted a multicenter, retrospective study based on 415 patients diagnosed with MDS or AEL according to the WHO 2008 classification. All patients had a bone marrow blast count below 20% of total nucleated cells. Patients were diagnosed at the VU University Medical Center ($n = 256$), a tertiary referral center in Amsterdam, and at secondary referral centers within The Netherlands, the Medical Center Leeuwarden ($n = 87$), Tjongerschans in Heerenveen ($n = 32$), Nij Smellinghe in Drachten ($n = 19$), Sint Antonius in Sneek ($n = 18$), and De Sionsberg in Dokkum ($n = 3$), between 2000 and 2013. Cytogenetic information was missing in 17% of the patients from the academic center and 40% of the patients from the secondary referral centers. In consequence, and because of unknown peripheral blood cell counts (mainly absolute neutrophil counts) in 10% and 3% of the patients, the revised International Prognostic Scoring System (IPSS-R) could not be employed in 27% and 43% of the patients from the academic center and secondary referral centers, respectively.[13] As might be explained by different referral patterns, patients diagnosed at the academic center were classified among higher WHO classification categories and IPSS-R risk groups, received different treatment regimens, and had a significantly younger age (Table 1 and Supporting Information Table S1). Furthermore, significantly more patients were diagnosed with AEL according to the WHO 2008 criteria at the academic center as compared to the secondary referral centers. In consideration of these dissimilarities, the impact of reclassification according to the WHO 2016 classification was first studied separately for the academic and secondary referral center cohorts. Since the analyses were comparable between the cohorts, the data of all 415 patients were presented combined.

The impact on our study population of the reclassification according to the WHO 2016 guidelines[4] is summarised in Supporting Information Figure S1. By elimination of the nonerythroid blast count rule, 30 patients previously considered with AEL according to the WHO 2008 criteria were now diagnosed with MDS. The majority of these patients were classified as MDS with excess blasts type 1 (MDS-EB-1, $n = 13$) or MDS with excess blasts type 2 (MDS-EB-2, $n = 14$). In addition, 3 patients were reclassified as MDS with multilineage dysplasia (MDS-MLD, $n = 2$) and MDS-U ($n = 1$). As shown by Kaplan Meier survival curves with the log-rank test, the overall survival time of MDS-EB patients with erythroid hyperplasia ($\geq$50% marrow nucleated erythroid cells) was comparable to patients lacking erythroid hyperplasia (19 months versus 17 months, Supporting Information Figure S2a). Surprisingly, MDS-EB patients with erythroid hyperplasia had a significantly longer leukaemia free survival time as compared to MDS-EB patients lacking erythroid hyperplasia (not reached *versus* 46 months, $P = .030$; Supporting Information Figure S2b). Of note, the leukaemia free survival time was defined as the number of months from diagnosis until the date of leukaemic progression. These findings are in line with the results of Bennett et al., who studied the value of the nonerythroid

TABLE 1 Clinical characteristics of the study population stratified by patient cohort

Characteristic	Tertiary referral center	Secondary referral centers	P value
Number of patients	256	159	
Median age (range)	66 (22–91)	75 (18–92)	<.001
Sex (male/female)	158/98	117/42	.013
WHO 2008 diagnosis			
MDS	230 (89.8%)	155 (97.5%)	.003
AEL	26 (10.2%)	4 (2.5%)	
WHO 2008 classification			
RCUD	15 (5.9%)	21 (13.2%)	.006
RARS	7 (2.7%)	16 (10.1%)	.002
RCMD	85 (33.2%)	24 (15.1%)	<.001
RAEB-1	36 (14.1%)	44 (27.7%)	.001
RAEB-2	63 (24.6%)	29 (18.2%)	.129
MDS-U	20 (7.8%)	17 (10.7%)	.317
MDS with isolated del(5q)	4 (1.6%)	4 (2.5%)	.492
WHO 2016 classification			
MDS-SLD	14 (5.5%)	19 (11.9%)	.018
MDS-MLD	47 (18.4%)	16 (10.1%)	.022
MDS-RS-SLD	8 (3.1%)	18 (11.3%)	.001
MDS-RS-MLD	37 (14.5%)	8 (5.0%)	.003
MDS with isolated del(5q)	6 (2.3%)	4 (2.5%)	.912
MDS-EB-1	47 (18.4%)	46 (28.9%)	.012
MDS-EB-2	76 (29.7%)	30 (18.9%)	.014
MDS-U	21 (8.2%)	18 (11.3%)	.290
IPSS-R			
Very low	10 (4.7%)	10 (10.4%)	.059
Low	55 (25.8%)	35 (36.5%)	.057
Intermediate	23 (10.8%)	23 (24.0%)	.006
High	48 (22.5%)	9 (9.4%)	.005
Very high	52 (24.4%)	14 (14.6%)	.042
Treatment			
Disease/ Immune MD	43 (20.0%)	31 (38.8%)	.001
Chemotherapy	83 (38.4%)	10 (12.5%)	<.001
Stem cell transplantation	61 (23.6)	0	<.001

Abbreviations: AEL: acute erythroid leukaemia; EB-1/-2: excess blasts type 1/2; IPSS-R: revised international prognostic scoring system; MD: modifying drugs; MDS-U: MDS unclassifiable; MDS: myelodysplastic syndromes; MLD: multilineage dysplasia; RAEB-1/-2: refractory anaemia with excess blasts type 1/2; RARS: refractory anaemia with ring sideroblasts; RCMD: refractory cytopaenia with multilineage dysplasia; RCUD: refractory cytopaenia with unilineage dysplasia; RS-SLD: ring sideroblasts with SLD; RS-MLD: ring sideroblasts with MLD; SLD: single lineage dysplasia.

blast count rule in a large series of MDS patients and did not find any impact on the leukaemia free survival and overall survival time.[14]

Of the 36 patients with refractory anaemia with unilineage dysplasia (RCUD), 33 patients remained classified into the same category, although referred to as MDS with single lineage dysplasia. Based on the presence of at least 15% ring sideroblasts and unilineage dysplasia other than dyserythropoiesis, 3 RCUD patients were reclassified as MDS with ring sideroblasts and unilineage dysplasia (MDS-RS-SLD). Of the 109 patients previously considered with refractory anaemia with multilineage dysplasia (RCMD), 2 patients were reclassified as MDS with isolated del(5q) syndrome since one additional cytogenetic abnormality besides del(5q) was found. The remaining 107 RCMD patients were separated into MDS-MLD ($n = 62$) and MDS with ring sideroblasts and multilineage dysplasia (MDS-RS-MLD, $n = 45$). Of note, the MDS-RS-SLD and MDS-RS-MLD categories were presumably underestimated due to lacking information on SF3B1 mutation status. As expected, the overall survival time and leukaemia free survival time for MDS-RS-MLD and MDS-MLD patients were not significantly different (Supporting Information Figure S3a,b).[15]

The MDS-U category remained unchanged in the WHO 2016 classification.[4] The major proportion of the MDS-U patients, i.e., 85%, was classified into this category based on the presence of 1% peripheral blood blasts. To study the rationale of this disease entity, the clinical outcome of MDS-U PB patients ($n = 33$) was compared with patients with less than 5% bone marrow blasts and no peripheral blood blasts ($n = 176$). Median overall survival of MDS-U PB patients as compared to patients without peripheral blood blasts was significantly shorter (31 *versus* 40 months, $P = .013$; Supporting Information Figure S4a). However, no difference in leukaemia free survival time was found (Supporting Information Figure S4b). The remaining MDS-U patients, i.e., 15%, fulfilled the MDS-U Pan criteria based on the presence of unilineage dysplasia and pancytopaenia. No difference in clinical outcome was found between MDS-U Pan patients and other MDS patients with less than 5% bone marrow blasts but no pancytopaenia (data not shown). This questions the reason behind the discrimination of MDS-U Pan patients as a separate category, which has not been argued by the WHO 2008 and WHO 2016 classification as well.[4,7]

The categories refractory anaemia with excess blasts type 1 and type 2 are now rephrased in the revised WHO classification. However, their diagnostic criteria remained unmodified, with preservation of the distinction between 5 to 9% and 10 to 19% bone marrow blasts, between 2 to 4% and 5 to 19% peripheral blood blasts, and between the absence or presence of Auer rods to distinguish the MDS-EB-1 from the MDS-EB-2 category.[4] Five years ago, the prognostic similarity of different levels of bone marrow blast counts in a range from ten to thirty per cent was shown by the introduction of the IPSS-R.[13] Also within this study population, patients classified as MDS-EB-1 or as MDS-EB-2 showed equal overall survival time and leukaemia free survival time (Supporting Information Figure S5a,b).

Finally, the prognostic value of the WHO 2016 classification was tested and compared to the IPSS-R.[4,13] As expected, the WHO 2016 classification and in addition the IPSS-R showed significantly prognostic value for the overall survival time as well as the leukaemia free survival time ($P < .001$, Supporting Information Figure S6a,b). Recently, we showed that morphological classification models for MDS have no auxiliary information beyond the IPSS-R, and are for that reason irrelevant from a prognostic point of view.[16,17] As confirmed by the likelihood ratio test in multivariate Cox regression analyses, also the current WHO 2016 classification had no prognostic value in addition to the IPSS-R in predicting the leukaemia free survival time and the overall

survival time. Vice versa, the IPSS-R showed significantly prognostic value additive to patients stratified by the WHO 2016 classification for both the overall survival time ($P < .001$) and the leukaemia free survival time ($P = .001$).

In summary, our data derived from multi-center expertise support the refinements made in the WHO 2016 classification for MDS. The WHO 2016 classification for MDS clearly squares up both difficult nomenclature and the puzzling nonerythroid blast count rule, and moreover, incorporates the progress made in elucidating the molecular pathogenesis. However, morphological assessment remains the cornerstone of MDS diagnosis, and certain features of the WHO 2016 classification for MDS are of questionable value.[4] Therefore, we propose some refinements to improve the understanding of current morphological classification for MDS:

1. The categories MDS-EB-1 and MDS-EB-2 may be merged into one single category.
2. The term MDS-U needs further clarification. As the name may imply this category is clearly not a leftover of unclassifiable MDS patients. To illustrate this, our data show that MDS-U PB patients have a worse outcome as compared to other MDS patients with less than 5% bone marrow blasts and no peripheral blood blasts.
3. The superior prognostic value of the IPSS-R as compared to the prognostic value of the WHO 2016 classification should be emphasised.

In conclusion, to achieve the original goal of the WHO, that is to establish a classification with both clear definitions and rationales, some refinements can be made in the WHO 2016 classification. We hope that our data can contribute to ongoing validation toward a more unambiguous morphological classification of MDS. Besides that, we and others emphasise the need for a more refined classification based on emerging new insights into the pathobiology of MDS. In this regard, understanding and addition of cytogenetic, molecular, immunophenotypic, and immunological features will improve the characterisation and, more important, therapeutic guidance of these heterogeneous disorders.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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Inflammatory and endothelial markers during vaso-occlusive crisis and acute chest syndrome in sickle cell disease

To the Editor:

Sickle cell disease (SCD) is characterized by chronic hemolytic anemia and recurrent vaso-occlusive crisis (VOC) that may lead to life-threatening acute complications such as acute chest syndrome (ACS). Pathophysiological factors contributing to VOC include endothelial activation, cell adhesion, coagulation activation and increased oxidative stress.[1] Even in sickle cell patients without symptoms of VOC a chronic inflammatory state is present as reflected by increased levels of circulating C-reactive protein (CRP), inflammatory cytokines, activated neutrophils, and increased levels of von Willebrand factor (VWF) antigen, indicating chronic endothelial activation. During VOC, an acute aggravation of ongoing inflammation with neutrophil and endothelial activation develops, whereby the exact trigger that initiates VOC and the sequence of events involved in VOC, including the possible progression to further acute complications such as ACS, are still incompletely understood.

In the current study, we aimed to gain insight in this sequence of events during VOC by assessing the dynamics in biomarkers of inflammation, cell death, neutrophil activation, and endothelial activation in patients with SCD during the course of hospitalization for VOC and the development of ACS. Levels of the acute phase proteins CRP and pentraxin3 (PTX3) were determined as markers of inflammation. Levels of the neutrophil derived azurophilic protein elastase in complex with its inhibitor α1-antitrypsin (HNE-α1-AT), and the neutrophil cytosolic protein calprotectin, a damage-associated-molecular-pattern protein (DAMP) that acts as an endogenous danger signal to support and aggravate an inflammatory response, were determined as neutrophil activation markers. Circulating nucleosomes, the basic units of DNA organization, were determined as a marker of systemic inflammation and cell death[2] and have been attributed to the formation of neutrophil extracellular traps (NETs) by activated neutrophils. Finally, VWF antigen and propeptide (VWF:Ag and VWFpp) were determined as markers of endothelial activation.

Consecutive adult patients with SCD (HbSS, HbSβ^0-thal, HbSβ^+-thal or HbSC) admitted for VOC to the Academic Medical Center or the Slotervaart Hospital (Amsterdam, The Netherlands) were approached for participation. Using a power analysis program an actual sample size of 32 patients was calculated.

Statistical data analysis was done with data of all included patients. Additional analyses were performed for patients with the relatively severe genotypes HbSS and HbSβ^0 (HbSS/HbSβ^0 group) and patients with the relatively milder HbSC and HbSβ^+ genotypes (HbSC/HbSβ^+ group) separately. Additional information on methods is available in the online supporting information.

Twenty-four sickle cell patients admitted for VOC were included in the study, accounting for 32 admissions (22 HbSS/Sβ^0 and 10 HbSC/Sβ^+). Baseline characteristics are presented in Supporting Information Table S1. Median hospital stay was 5.5 (IQR 3–8) days for the whole group. Five patients (4 HbSS and 1 HbSβ^+ patient) developed an ACS during admission. All patients with ACS received antibiotics and two patients (both HbSS) required erythrocytapheresis. The presence of a respiratory viral infection as trigger for VOC was ruled out in all patients that were tested (27 out of 32 admissions) using multiplex RT-PCR.

During hospitalization the different biomarkers showed clearly distinct temporal patterns (Figure 1 and Supporting Information Table S2). Levels of CRP and PTX3 and were significantly elevated and respectively peaked on day 2 and 3 of the VOC indicating that VOC is accompanied by an acute inflammatory response. Since CRP is considered to be a reflection of systemic inflammation in response to interleukin-6 and PTX3 levels mainly reflect local inflammation, as it is released locally by neutrophils and endothelial cells,[3] both forms of inflammatory response seem to be active in VOC. Nucleosome levels peaked at day 1 followed by a rapid decline the days thereafter and mimicked the course of HNE-α1-AT (Figure 1C,D). Calprotectin levels did not significant change compared to steady state in the analysis of all patients. (Figure 1E). Interestingly, plasma levels of all inflammatory biomarkers and in particular markers of neutrophil activation were significantly higher in patients developing ACS. (Figure 1)

Previously, levels of nucleosomes, HNE- α1-AT[4] and PTX3[5] have been demonstrated to relate with clinical severity. Our current data support these findings, given the fact that the highest levels of nucleosomes, HNE-α1-AT and calprotectin were observed in patients that developed an ACS (Figure 1). Exchange transfusion early in the onset of ACS is considered best treatment, but identifying patients at risk for ACS development is challenging. While only modest elevation of HNE-α1-AT and calprotectin levels in our patients with VOC were observed in this study, very high levels were found in the patients developing ACS. It would be interesting to investigate the value of these markers prospectively to identify patients at risk for developing ACS in future studies.

Plasma levels of VWF:Ag were increased in the absence of elevated VWFpp and remained stable during the course of VOC and were