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The Placement of Auer Rods as a Single Diagnostic Criterion for the Highest-Risk Category of Myelodysplastic Syndromes: A Single-Institution Study and Critical Analysis

Rina Kansal[✉,1,2,*]

[1] Molecular Oncology and Genetics, Diagnostic Laboratories, Versiti Blood Center of Wisconsin, Milwaukee, WI 53233, USA
[2] Department of Pathology and Anatomical Sciences, The University at Buffalo, Buffalo, NY 14260, USA

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Abstract
Accurate determination of prognostic risk is critical for patients and treating clinicians. The French-American-British classification of myelodysplastic syndromes (MDS) described refractory anemia with excess blasts (RAEB) in transformation (RAEB-T), including two subsets with <20% bone marrow (BM) blasts, which were reclassified as RAEB-2, the highest-risk MDS category in the 2001 World Health Organization classification. Those diagnostic criteria have been retained until now with nomenclature changes from RAEB-2 to "MDS with excess blasts-2" in 2017 and "MDS with increased blasts-2" (MDS-IB2) in 2022. The placement of Auer rods (AR) in the diagnostic classification has been controversial since its inception. This single-institution retrospective study analyzed 22 consecutive *de novo* MDS patients with <20% BM blasts originally diagnosed as RAEB-T only due to AR (n = 13) or ≥5% peripheral blood (PB) blasts ± AR (n = 9), applying the International Prognostic Scoring System (IPSS) and revised-IPSS (IPSS-R) for MDS. The AR-positive group showed 67% males, younger age (p = 0.01), median 8.4% (range 2.6–15.0) BM blasts, 58% normal (0% complex) karyotypes, and low/int-1 IPSS in all (100%) patients with <5% BM blasts compared with the ≥5% PB blast group with 55% females, median 10.5% (range 5.4–15.0) BM blasts, 67% abnormal (17% complex) karyotypes, and high/very high-risk IPSS-R (83%). Auer rod-positive MDS was associated (p = 0.01) with BM transplant referral/procedure but not (p = 0.88) with acute leukemia development. This small study suggests that Auer rod-positive MDS is likely distinct from Auer rod-negative MDS and genetically from MDS with ≥5% PB blasts. A critical analysis shows high-risk IPSS-R scores in previously reported patients with AR-positive MDS and <5% BM blasts, confirming that IPSS-R variables, not Auer rods, accounted for their adverse outcomes. Despite recent studies, genomic features of Auer rod-positive MDS patients with low blast percentages are unknown. No rationale is identified in this study and analysis for placing Auer rods as a diagnostic criterion for the highest-risk diagnostic category of MDS. Additional studies are warranted incorporating genomic analyses and other clinical, hematologic, and genetic variables, specifying BM blast percentages, to ascertain if Auer rods have any place in the diagnostic classification of MDS.

Keywords:
myelodysplastic syndromes; bone marrow diseases; cancer; diagnosis; prognosis; classification; genetics; neoplasms

1. Introduction

The myelodysplastic syndromes (MDS) are a heterogeneous group of myeloid neoplasms characterized by ineffective hematopoiesis, peripheral blood cytopenia, and a variable propensity to develop acute myeloid leukemia (AML). A unique feature in many patients with MDS is the paradox of cytopenias despite having a cellular bone marrow [1]. These diseases primarily affect older adults, with 86% of MDS diagnosed in patients ≥ 60 years in the USA, with a median age at diagnosis of 76 years [2], and 79.5% of MDS diagnosed in Japanese patients older

* **Corresponding Author:**
Rina Kansal, Department of Pathology, University of Michigan, Ann Arbor, MI 48109, USA, rinakansal@msn.com or rkansal@buffalo.edu

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than 65 years [3]. The incidence of MDS increases with advancing age, reaching its peak around 70 years, and the disease occurs more frequently in males than females [4]. The age-adjusted incidence in men is higher among males (4.4 per 100,000) than females (2.5 per 100,000) in the USA [5]. Most MDS occur *de novo*, i.e., without a known cause. However, myelodysplastic syndromes also occur secondary to exposure to ionizing radiation, previous chemotherapy, chemicals such as benzene, and hereditary diseases [6], including in pediatric and adult patients with a germline predisposition to malignancy (reviewed in [7,8]). About 30% of patients with MDS progress to acute myeloid leukemia [9], a hematologic malignancy that is fatal if untreated. The risk of developing an AML is higher in patients with high-risk MDS than in patients with low-risk MDS; that risk is determined most widely by the International Prognostic Scoring System (IPSS) initially developed in 1997 [10] and the revised IPSS (IPSS-R) in 2012 [11]. Molecular genetic abnormalities in MDS were incorporated into the IPPS in 2022, and this update improved upon the IPSS-R [12]. However, advanced molecular genetic analysis is not yet widely accessible outside specialized centers. Even when available, the results often require several weeks, emphasizing the continued necessity and reliance on morphologic evaluation as the primary method for diagnosing and prognosticating MDS [13]. Significantly, the treatment goals and algorithms for patients with MDS differ according to the level of prognostic risk as determined by the prognostic systems, as described in the European LeukemiaNet guidelines [14] and the current National Comprehensive Cancer Network Guidelines [15].

Historically, Auer rods were described and illustrated by John Auer in 1906 as a "refractile, rod-like body, 1 to 6 microns in length and up to 0.2 microns in thickness." Auer noted that these structures "stained red with methylene azure stains, occasionally with a bluish tinge" and were present in the cytoplasm of large lymphoid cells in a 21-year-old male patient with acute leukemia [16]. In that publication, Auer acknowledged Thomas McCrae for providing the clinical notes for that patient, described by McCrae as patient number 4 in an earlier publication for five patients with acute lymphoblastic leukemia in 1905 [17]. The correct nature of Auer rods being present in myeloid cells instead of lymphoid cells was established in 1917 when the "Auer bodies" were shown to stain with the oxidase reaction in immature myeloid cells in acute myeloid leukemia [18]. In 1995, Seymour and Estey described how prominent hematologists in the early part of the 20th century strongly associated Auer rods with acute leukemias, and that association remained strong until half a century later when the French American British (FAB) group described their classification of acute leukemias and MDS [19].

Myelodysplastic syndromes were considered "pre-leukemic" diseases (reviewed in [9,20]) before the term "myelodysplastic disease" was first suggested by Harriet Gilbert in New York in 1970 (reviewed in [20]). In 1976, the FAB Group proposed their initial classification of acute leukemias, which were widely accepted at that time as requiring cytotoxic chemotherapy at diagnosis [21]. In that same paper, two broad categories of dysmyelopoietic syndromes were described as "a range of conditions for which immediate initiation of therapy cannot be recommended or may not be indicated" [21]. In 1982, the FAB group used the term "myelodysplastic syndromes" (MDS) for these diseases and described five types of MDS: refractory anemia (RA), refractory anemia with ringed sideroblasts (RARS), refractory anemia with excess of blasts (RAEB), RAEB in transformation (RAEB-T), and chronic myelomonocytic leukemia (CMML) [22]. They defined AML as having >30% bone marrow blasts, RAEB as having >5 to <20% bone marrow blasts and <5% peripheral blood blasts, and the highest risk MDS category, RAEB-T, as having (1) 20–30% bone marrow blasts, or (2) >5% peripheral blood blasts, or (3) the presence of Auer rods in the peripheral blood or granulocytic precursors in the bone marrow [22,23]. Several prognostic studies in MDS were then performed, which led to the International MDS Risk Analysis Workshop in 1994 and the publication of the IPSS in 1997, which recognized the significance of cytogenetics, bone marrow blast percentages, and the numbers of cytopenias in determining prognostic risk [10].

In 2001, the previously separate classifications of myeloid and lymphoid neoplasms were integrated into a single diagnostic World Health Organization (WHO) classification (reviewed in [24]), published as the third edition of the WHO "Blue Book" [25]. The 2001 WHO classification made the following significant changes that affected the classifications of AML and MDS [25–27]: (1) The blast percentage required for a diagnosis of AML was lowered from 30% to 20%, except for three types of AML with the genetic abnormalities, t(15;17), t(8;21), and inv(16) considered sufficient to diagnose AML irrespective of blast percentage; (2) The category of RAEB-T was eliminated, and RAEB-T's most significant subset comprised of >20% bone marrow blasts was classified as AML; (3) The RAEB category was split into two, RAEB-1 and RAEB-2, defined by bone marrow blast percentages of 5–10% and 10–20%, respectively, based on data published by the International MDS Risk Analysis Workshop [10]. Germing et al. validated the WHO proposals in a large study in 2000 [28], wherein it was considered appropriate to reclassify patients with RAEB-T into RAEB-1

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or RAEB-2 according to the bone marrow and peripheral blood blast counts; they also stated in their paper that "the presence of Auer rods can no longer be considered a bad prognostic marker" [28]. The WHO 2001 classification included the two smaller subsets of RAEB-T defined only by the presence of Auer rods or ≥5% peripheral blood blasts in RAEB-2 [25–27]. The WHO 2001 book stated that "the significance of the detection of Auer rods is not completely clear," along with the recommendation to classify patients with RAEB and Auer rods as RAEB-2 [25] (p. 71). The fourth edition of the WHO classification of hematolymphoid tumors was published in 2008 [29,30] and revised in 2017 [31,32]. The fifth edition update of the WHO classification of myeloid neoplasms was published as a summary paper in 2022 [33], and the online beta version of the complete volume became available on the WHO Tumor Classification Books website in August 2022 [34]. The above-described changes in the classification of MDS from the FAB classification to the fifth edition of the WHO classification in 2022 are depicted in Figure 1.

The current 2022 WHO classification of MDS in adults includes three types defined by genetic findings and three types defined by morphologic features, as shown in Figure 2. This figure also shows the parallel International Consensus Classification of MDS published in 2022 by a group independent from the WHO classification [35]. Two types of pediatric MDS are recognized by morphology separately from MDS in adults [33–35].

Notably, the WHO classification of Tumors is used worldwide for all types of tumors, including in countries and geographic regions with limited resources for the diagnosis of tumors. The fifth edition of the WHO classification is designed to be evidence-based [36]. Of note, the fifth edition of the WHO classification "favored a classification of MDS based on comprehensive risk-stratification schemes such as the IPSS-R for MDS" as a factor to enhance rigor in classification [33,34].

To date, the WHO classification has maintained the backbone of the FAB classification of MDS, as described in 2023 [37]. This is especially true in the types of MDS in adult patients having the highest prognostic risk for whom the WHO diagnostic criteria have been unchanged since the inclusion of RAEB-2 in the WHO classification in 2001 to the renaming of RAEB-2 as "MDS with excess blasts-2" in 2017 [32], and "MDS with increased blasts-

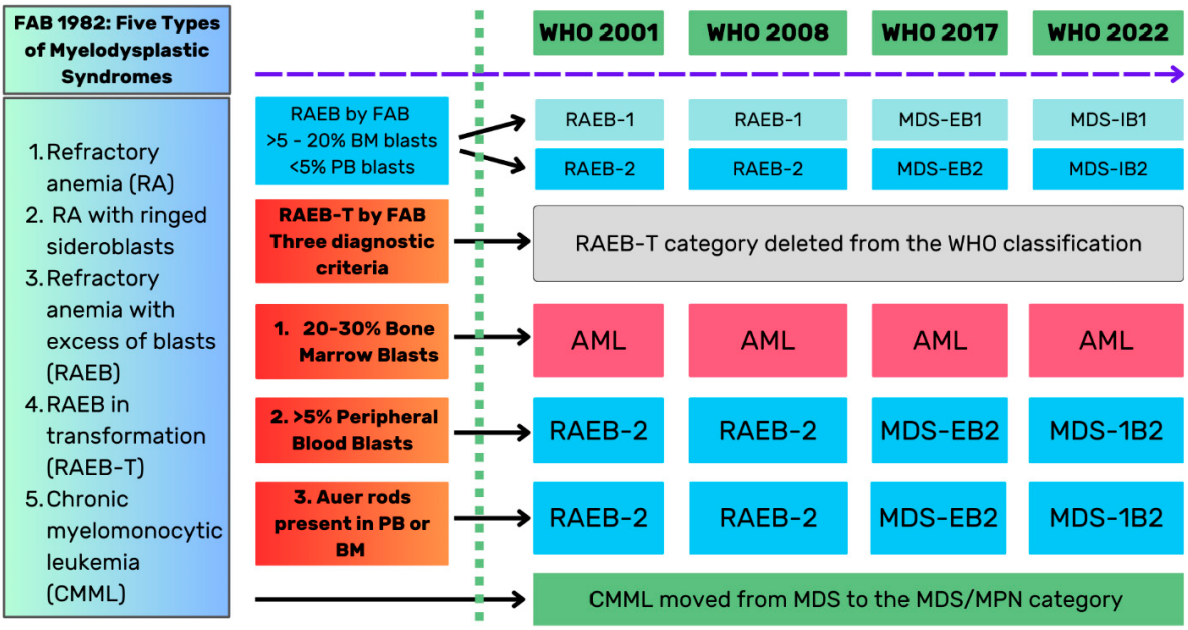


Figure 1: This figure depicts the five types of myelodysplastic syndromes described by the FAB classification in 1982 [22] and the changes made in the FAB categories of refractory anemia with excess of blasts (RAEB) and RAEB in transformation (RAEB-T) by the World Health Organization (WHO) classification in 2001 [25–27], which were retained in the updates of the WHO classification in 2008 [29,30], 2017 [31,32], and 2022 [33,34], except for terminology changes from RAEB-2 to MDS with excess blasts-2 (MDS-EB2) in WHO 2017 and MDS with increased blasts-2 (MDS-IB2) in the WHO 2022 classification. Abbreviations: FAB, French-American-British; WHO, World Health Organization; RAEB, refractory anemia with excess of blasts; RAEB-T, refractory anemia with excess of blasts in transformation; BM, bone marrow; PB, peripheral blood; RAEB-1, refractory anemia with excess blasts-1; RAEB-2, refractory anemia with excess blasts-2; MDS-EB1, MDS with excess blasts-1; MDS-EB2, MDS with excess blasts-2; MDS-IB1, MDS with increased blasts-1; MDS-IB2; MDS with increased blasts-2; AML, acute myeloid leukemia; MDS/MPN, myelodysplastic syndrome/myeloproliferative neoplasm.

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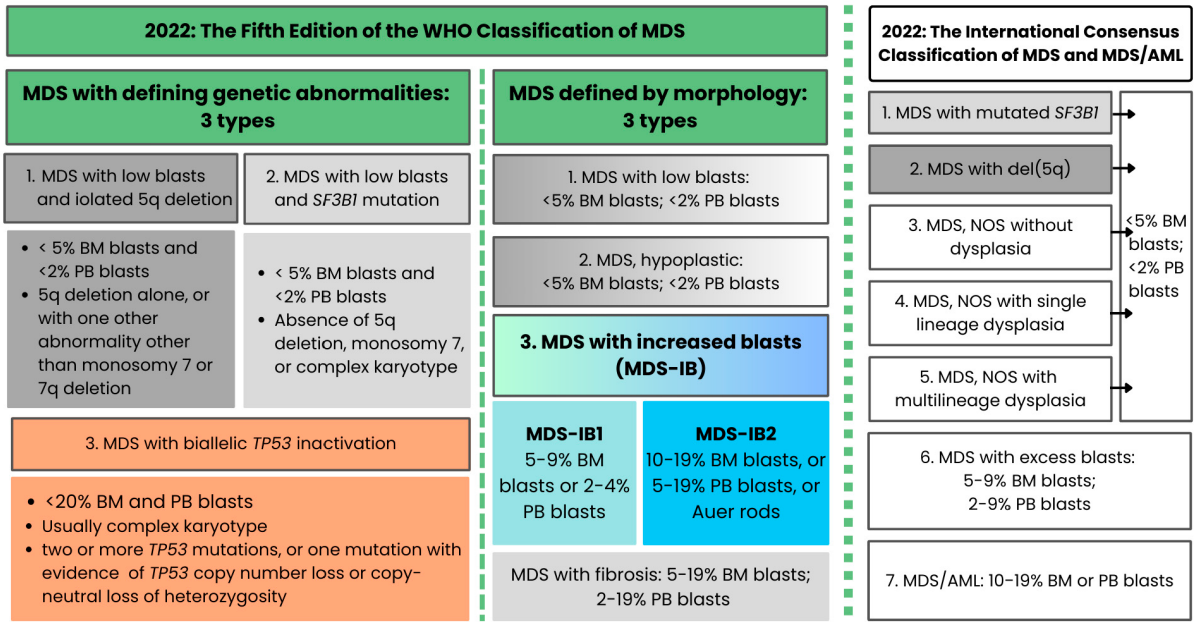


Figure 2: The left part of this figure depicts the three types of MDS defined by genetics and the three types defined by morphologic evaluation in the WHO 2022 classification [33,34]. MDS defined by morphology includes MDS with increased blasts-1 (MDS-IB1) and MDS with increased blasts-2 (MDS-IB2), with the latter including Auer rods as a sole diagnostic criterion [33,34], similar to the revised fourth edition of the WHO classification published in 2017 [32]. The main types of MDS and MDS/AML defined by the International Consensus Classification (ICC) in 2022 are shown in the right part of the figure [35]. Abbreviations: WHO, World Health Organization; MDS, myelodysplastic neoplasms or myelodysplastic syndromes; BM, bone marrow, PB, peripheral blood; MDS-IB1, MDS with increased blasts-1; MDS-IB2, MDS with increased blasts-2; NOS, not otherwise specified; MDS/AML, MDS/acute myeloid leukemia category in the ICC [35].

2" (MDS-IB2) in the fifth edition in 2022 [33,34]. The fourth edition of the WHO classification in 2008 included the sole presence of Auer rods as diagnostic of this highest-risk MDS category, even in patients with <5% bone marrow blasts [29] (p. 91) based on a multi-institutional publication by Willis et al. that showed heterogeneous, adverse patient outcomes in selectively identified patients with Auer rods and low blasts [29,38]. Willis et al. initiated their study following the author's presentation of preliminary findings from a single institution study at the Society for Hematopathology's scientific platform session at the United States and Canadian Academy of Pathology annual conference in 2001 [39]. They derived support from the earlier presented findings based only on IPSS scores, as discussed in their publication in 2005 [38]. The understanding of MDS has substantially increased since 2001, especially since 2011, with studies of molecular genetics in MDS [40–52], MDS with *SF3B1* mutations as a distinct disease [46,48], clonal hematopoiesis as a preneoplastic precursor for hematologic malignancies [53–57], and germline predisposition to MDS [58], including due to germline *DDX41* variants [59–61] (reviewed in [7]). Further, there has been an increased depth of prognostic risk stratification by IPSS-R for over a decade since 2012 [11]. In addition, two recent reports have studied Auer rods in patients with MDS in the context of the current understanding of the underlying molecular genetics in MDS [62,63]. Considering the present knowledge of the biology of MDS, this work focuses on the inclusion of Auer rods as a single diagnostic criterion for the highest-risk category of MDS, which has been discussed controversially since its introduction in the FAB classification [19,25,64–66]. This paper presents the results of the original single-institution study performed at the University of Michigan and a critical review of current literature to synthesize the evidence, if any, regarding the rare but essential subset of patients diagnosed with MDS-IB2 solely due to the presence of Auer rods in the current WHO classification, despite the absence of 10–19% bone marrow blasts.

2. Materials and Methods

2.1. Study Initiation and Design

During routine hematopathology practice at the University of Michigan, the author had identified rare, unequivocal Auer rods in two blasts in bone marrow aspirate smears from a patient with isolated neutropenia and less than 5%

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bone marrow blasts. One of two repeated bone marrow biopsies in that patient again showed rare, unequivocal Auer rods and < 5% bone marrow blasts, and the patient was scheduled for a bone marrow transplant (BMT). The pre-transplant bone marrow biopsy unexpectedly showed features that were diagnostic of AML. The findings in this index patient led to studying the two smaller groups of RAEB-T patients with < 20% bone marrow blasts, excluding patients with 20–30% bone marrow blasts. The University of Michigan Institutional Review Board approved this retrospective study.

2.2. Cases Retrieved

The surgical pathology database at the University of Michigan was searched to identify consecutive patients diagnosed with RAEB-T over five years (1995 to June 2000). The significant subset comprising RAEB-T with 20–30% bone marrow blasts representing AML by the WHO classification was excluded. The pathology slides and reports for the remaining RAEB-T cases with < 20% bone marrow blasts were retrieved. The author reviewed all pathology slides and reports. The diagnosis of MDS was confirmed in all patients after reviewing bone marrow trephine biopsy hematoxylin and eosin (H&E)-stained sections and the corresponding Wright-Giemsa-stained bone marrow aspirate and peripheral blood smears, in conjunction with a review of the clinical history and laboratory findings present in the University of Michigan medical records, after excluding non-MDS causes of cytopenias and hematologic dysplasia. The diagnosis of RAEB-T by FAB criteria was confirmed with at least 500-cell bone marrow differential counts and 200-cell peripheral blood blast counts. At least one unequivocal Auer rod in peripheral blood or bone marrow aspirate smears was required for Auer rod positivity.

Five patients were excluded: (1) one due to unavailable marrow aspirate smears, (2) one classified as CMML in transformation, and (3) one with 3% bone marrow blasts and Auer rods with the subsequent detection of the t(8;21) translocation diagnostic of AML with a recurrent cytogenetic abnormality by the WHO 2001 classification, based on the Airlie House report published by then [26]; this patient with the t(8;21) translocation progressed to having 17% bone marrow blasts within two months. (4) Two additional patients, one male and one female, with >5% peripheral blood blasts and no Auer rods, were excluded due to a previous history of receiving cytotoxic therapy. Bone marrow blast percentages in these two excluded patients were in the 5–10% group in one and the 10–20% group in the other; karyotypic analyses in both patients showed a complex karyotype with more than three cytogenetic abnormalities, including monosomy 7 in both patients. Twenty-two patients with *de novo* MDS having less than 20% bone marrow blasts were identified in 2000 for this study, all initially diagnosed as RAEB-T due either to the presence of Auer rods or ≥ 5% peripheral blood blasts with or without Auer rods.

2.3. Cytogenetics

Cytogenetic analyses of bone marrow aspirate samples were performed at the University of Michigan. When possible, twenty metaphases were examined in the cytogenetics laboratory. The criteria defined by the International System for Human Nomenclature, 1995 [67], were used for the identification of clonal abnormalities. A clonal abnormality was required to have at least two cells with the same aberration. If the abnormality was a missing chromosome, the same change was required to be present in at least three cells to be accepted as clonal [67] (p. 78). The cytogenetic abnormalities were recorded from the reports and classified according to the IPSS [10] as follows: 'good,' normal, -Y, del(5q), del(20q); 'poor,' complex (≥ 3 abnormalities); 'intermediate,' all other abnormalities [10]. Subsequently, these findings were grouped into the five prognostic subgroups as per the IPSS-R [11], as follows: 'very good,' -Y, del(11q); 'good,' normal, del(5q), del(12p), del(20q), double including del(5q); 'intermediate,' del(7q), +8, +19, i(17q), any other single or double independent clones; 'poor,' −7, inv(3)/t(3q)/del(3q), double including −7/del(7q), complex: three abnormalities; 'very poor,' complex: > three abnormalities [11].

2.4. Clinical Findings

The author reviewed the medical records for the clinical history and laboratory findings and recorded the following parameters: previous history of cytotoxic therapy, patient age, sex, hematologic parameters at diagnosis {hemoglobin, total white cell count (WBC), absolute neutrophil count (ANC), platelet count}, cytogenetic (karyotypic) findings, and the available clinical follow-up until May 2001. The IPSS and IPSS-R scores and the corresponding risk categories were calculated from the retrieved data, as described [10,11]. In the IPSS, cytopenias were defined as hemoglobin <10 g/dL, ANC <1.5 × 10^9/L, and platelet count <100 × 10^9/L [10]. The IPSS-R accounts for the depth of cytopenias, with hemoglobin values separated as <8 g/dL, 8 to <10 g/dL, and ≥10 g/dL, ANC cut-off at >0.8 × 10^9/L, and platelet counts separated as <50 × 10^9/L, 50 to <100 × 10^9/L, and ≥100 × 10^9/L [11]. Also, the IPSS-R defines scores for five prognostic risk categories: ≤1.5 very low, >1.5 to 3 low, >3 to 4.5 intermediate, >4.5 to 6 high, and >6 very high

risk [11], instead of the following four IPSS categories: 0 low, 0.5 to 1.0 int-1, 1.5 to 2.0 int-2, ≥2.5 high [10], as shown in Figures 3 and 4.

Figure 5 compares the clinical outcomes between the IPSS and IPSS-R and is adapted from the two publications [10,11].

2.5. Patients Included According to the Fifth Edition of the WHO Classification in 2022

Additional cases were excluded from the retrieved groups of patients according to the WHO 2022 classification to focus on patients with less than 10% bone marrow blasts classified as MDS-IB2 only due to the presence of Auer rods or ≥5% peripheral blood blasts.

2.6. Statistical Analysis

A χ^2 test of independence and the Mann-Whitney U rank score test [68], respectively, was performed to determine if there was an association between the patient's sex and age at the time of diagnosis and the presence or absence of Auer rod-positive disease. In addition, the Mann-Whitney U test was performed separately for each variable to determine any association of hemoglobin, WBC, and platelet counts with Auer rod-positive or Auer rod-negative disease. χ^2 tests of significance were also performed to determine any association between the presence of Auer rods and the development of acute leukemia, and separately for any association between the presence of Auer rods and a referral for a bone marrow transplant. All statistical tests were performed using online calculators at the accessible Social Science Statistics website [69]. All p-values were two-sided. A p-value of <0.05 was considered significant.

3. Results

The results are organized into two main sections, with the first (Section 3.1) describing the 22 patients with *de novo* MDS diagnosed as RAEB-T by the FAB classification. Section 3.2 describes the patients after classifying according to the fifth edition of the WHO classification.

3.1. Twenty-Two Patients with De Novo MDS Diagnosed as RAEB-T with <20% Blasts by the FAB Classification

Table 1 shows the distribution of the 22 patients with *de novo* MDS having less than 20% bone marrow blasts diagnosed as RAEB-T by the FAB classification [22] only due to the presence of Auer rods (n = 13) or ≥5% peripheral blood blasts ± Auer rods (n = 11). Table 2 shows the patient demographics. These tables are included to allow comparison with the earlier studies published before the WHO 2008 classification.

Three Prognostic Variables in the IPSS	Values Are Assigned for Each of the Three Variables: Range 0 to 2.0					IPSS Risk Groups Are Defined by the Total Scores
	0	0.5	1.0	1.5	2.0	
Bone Marrow Blasts %	<5	5-10	-	11-20	21-30	**Total Scores:** Low Risk = 0 INT-1 Risk = 0.5 - 1.0 INT-2 Risk = 1.5 - 2.0 High Risk = 2.5 or greater
Karyotype	Good	Inter-mediate	Poor	-	-	
Cytopenias	0/1	2/3	-	-	-	

Karyotypes:
Good = Normal, -Y, del(5q), del(20q);
Poor = complex (3 or more abnormalities) or chromosome 7 abnormalities;
Intermediate = other abnormalities

Figure 3: The International Prognostic Scoring System (IPSS) for Myelodysplastic Syndromes shows the prognostic variables, the numerical values assigned to each variable, and the risk scores and risk groups based on the total values combined from values for each variable. Cytopenias were defined as hemoglobin <10 g/dL, absolute neutrophil count <1.5 × 10^9/L, and platelet count <100 × 10^9/L [10]. This Figure is adapted from the cited publication by Greenberg et al. 1997 [10].

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Five Prognostic Variables in the IPSS-R	Values Are Assigned for Each of the Five Variables: Range 0 to 4						
	0	0.5	1	1.5	2	3	4
Cytogenetics	Very Good	-	Good	-	Inter-mediate	Poor	Very Poor
Bone Marrow Blasts %	2% or less	-	>2% to <5%	-	5% -10%	> 10%	-
Hemoglobin	10 or greater	-	8 to < 10	< 8	**IPSS-R Risk Groups are Defined by the Total Scores:** Very Low Risk = 1.5 or less Low Risk = > 1.5 to 3 Intermediate Risk = > 3 to 4.5 High Risk = > 4.5 to 6 Very High Risk = > 6		
Platelets	100 or greater	50 to < 100	< 50	-			
Absolute Neutrophil Count	0.8 or greater	< 0.8	-	-			

Figure 4: The Revised International Prognostic Scoring System for Myelodysplastic Syndromes (IPSS-R) shows the prognostic variables, the numerical values assigned to each variable, and the risk scores and risk groups based on the total values combined from values for each variable. The five prognostic cytogenetic subgroups were defined as follows: 'very good,' -Y, del(11q); 'good,' normal, del(5q), del(12p), del(20q), double including del(5q); 'intermediate,' del(7q), +8, +19, i(17q), any other single or double independent clones; 'poor,' −7, inv(3)/t(3q)/del(3q), double including −7/del(7q), complex: three abnormalities; 'very poor,' complex: > three abnormalities [11]. The hemoglobin values were separated as <8 g/dL, 8 to <10 g/dL, and ≥10 g/dL, absolute neutrophil count cut-off at >0.8 × 10^9/L, and platelet counts separated as <50 × 10^9/L, 50 to <100 × 10^9/L, and ≥100 × 10^9/L [11]. This Figure is adapted from the cited publication by Greenberg et al. 2012 [11].

Clinical Outcomes in IPSS Risk Groups	Clinical Outcomes in IPSS-R Risk Groups
Median survival in IPSS Risk Groups Low Risk = 5.7 years Int-1 Risk = 3.5 years Int-2 Risk = 1.2 years High Risk = 0.4 years	**Median survival in IPSS-R Risk Groups** Very Low Risk = 8.8 (7.8 - 9.9) years Low Risk = 5.3 (5.1 - 5.7) years Intermediate Risk = 3.0 (2.7 - 3.3) years High Risk = 1.6 (1.5 - 1.7) years Very High Risk = 0.8 (0,7 - 0.8) years
25% Patients Evolve to AML (years) Low Risk IPSS = 9.4 years Int-1 Risk IPSS = 3.3 years Int-2 Risk IPSS = 1.1 years High Risk IPSS = 0.2 years	**25% Patients Evolve to AML (years)** Very Low Risk IPSS-R = NR (14.5 - NR) years Low Risk IPSS-R = 10.8 (9.2 - NR) years Intermediate Risk IPSS-R = 3.2 (2.8 - 4.4.) years High Risk IPSS-R = 1.4 (1.1 - 1.7) years Very High Risk IPSS-R = 0.73 (0.7 - 0.9) years

Figure 5: Comparison of the clinical outcomes between the International Prognostic Scoring System (IPSS) and the Revised IPSS (IPSS-R) for Myelodysplastic Syndromes. This Figure is adapted from two cited publications [10,11]. Abbreviations: AML, acute myeloid leukemia; NR, not reached.

Table 1: Distribution of 22 patients with *de novo* MDS having <20% bone marrow blasts diagnosed as RAEB-T only due to Auer rods or ≥ 5% peripheral blood blasts.

Bone Marrow Blasts %	Numbers (%) of RAEB-T Patients Due To Having Auer Rods Alone or ≥5% Peripheral Blood Blasts by the FAB Classification		
	Auer Rods as the Only Criterion	≥5% Blasts in Peripheral Blood	
		Auer Rods Negative	Auer Rods Positive
Less than 5%	3 [a] (14%)	0 (0%)	0 (0%)
5 to 19%	10 (45%)	7 (32%)	2 (9%)
5 to <10%	6 (27%)	3 (14%)	1 (4.5%)
≥10 to 19%	4 [b] (18%)	4 (18%)	1 (4.5%)
Less than 20%	**13 (59%)**	**7 (32%)**	**2 (9%)**

Abbreviations: RAEB-T, refractory anemia with excess of blasts in transformation; FAB, French American British classification; [a] One patient had three bone marrow biopsies, two of which showed Auer rods and <5% bone marrow blasts; [b] Two patients had a reported clinical history of MDS. The numbers (%) in the bottom row in bold font represent the total numbers (%) of patients in each column group.

Table 2: Patient demographics in the two RAEB-T subgroups having < 20% bone marrow blasts and either only Auer rods or ≥ 5% peripheral blood blasts with or without Auer rods.

	All RAEB-T Patients with <20% BM Blasts	Auer Rods as the Only Defining Criterion			≥5% PB Blasts with or Without Auer Rods		
		<5% BM blasts	5–19% BM blasts	<20% BM blasts	Auer rods negative	Auer rods +	Auer rods ±
Patients N	22	3	10	13	7	2	9
Age, median (range) years	60 (20–85)	44 (20–58)	60 (38–79)	59 (20–79)	69 (54–85)	34, 58	62 (34–85)
Male: Female	14:8	2:1	8:2	10:3	4:3	0:2	4:5

Abbreviation: RAEB-T, refractory anemia with excess of blasts in transformation; BM, bone marrow; PB, peripheral blood; "+" positive; "±" positive or negative.

The ages of patients at diagnosis and the male-to-female ratios for all patient sub-groups are shown in Table 2.

These 22 patients included ten males and five females in the Auer rod-positive group, including two Auer rod-positive females in the ≥5% peripheral blood blast group. Four males and three females comprised the Auer rod-negative group. Male dominance was present in the Auer rod-positive group, with a male-to-female ratio of 2:1. However, a chi-square test of independence showed no significant association between the patient's sex and the presence of Auer rods: X^2 (2, N = 22) = 0.1871, p = 0.665362.

In contrast, younger age was significantly associated with the presence of Auer rods in this study compared with the Auer rod-negative group of patients (p = 0.0198). The 15 patients in the Auer rod-positive group, including the two with ≥5% peripheral blood blasts, were significantly younger than the seven Auer rod-negative patients with MDS, who had ≥5% peripheral blood blasts and 5–19% bone marrow blasts.

3.1.1. Hematologic, Cytogenetic, and Clinical Findings Related to the IPSS and IPSS-R Prognostic Scores in all RAEB-T Patients with <20% Bone Marrow Blasts, *N* = 22

All three blood counts (hemoglobin, WBC, and platelets) were available in 17 (77%) patients; one or two counts were available in two patients. Of the three patients with unavailable counts, two had transfusion-dependent anemia, and one of these two was also platelet transfusion-dependent. Peripheral blood cytopenia, as defined by the IPSS, was present in all 21 (100%) with available counts, with at least one cytopenia in 8 (36%) and at least two cytopenias in 13 (59%) patients. Hemoglobin values were ≥10 g/dL in five (21%), 8–10 g/dL in eight (36%), <8 g/dL in at least five (22%), and unavailable in four (18%), including three patients with anemia, two of whom were transfusion dependent. The WBC counts were 1.1–20.9 (median 4.2) × 10^9/L in 17 (77%), with ANC 0.2–2.1 × 10^9/L in nine (41%), and <0.8 × 10^9/L in one (4.5%). The ANC values were unavailable in twelve (45%) patients with WBC counts ranging from 1.8–20.9 (median 7.4) × 10^9/L, including in the three patients with

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anemia and unavailable hemoglobin values mentioned in this paragraph. The platelet counts were 10–266 (median 84) $\times$ 10^9/L in 18 (82%), >100 $\times$ 10^9/L in 8 (36%), 50–100 $\times$ 10^9/L in four (18%), <50 $\times$ 10^9/L in six (27%), and unavailable in four (18%).

Bone marrow blast percentages ranged from 2.6%–15.0% (median 9.1%) for all 22 patients. Results of cytogenetic (karyotypic) analyses were available in 18 (82%) of 22 patients, including 14 Auer rod-positive and four Auer rod-negative patients. The 14 Auer rod-positive patients included the two patients with ≥5% peripheral blood blasts. The karyotypes were normal in nine (50%) of 18 patients, including eight Auer rod-positive and one Auer rod-negative patients. One of these patients had t(2;3)(p23;q29) as a constitutional abnormality and an otherwise normal karyotype with no clonal abnormality. The nine patients with normal karyotypes were aged 20 to 84 years, with a median age of 58. These nine patients included six males and three females, with bone marrow blast percentages ranging from 2.6% to 15% and a median of 6.9%.

A clonal cytogenetic abnormality was present in nine (50%) of 18 patients, including six Auer rod-positive and three Auer rod-negative patients. Trisomy 8 was the most common karyotypic abnormality in this cohort and was present in five patients; three of these patients were Auer rod-positive, and two were Auer rod-negative. Trisomy 8 was the sole abnormality in three of these five patients, two of whom were Auer rod-positive and the third Auer rod-negative. The other two patients with trisomy 8 harbored additional clonal karyotypic abnormalities. Trisomy 11 was present in addition to trisomy 8 in one Auer rod-positive patient. In the other, an Auer rod-negative patient, trisomy 8 was present with a derivative of chromosomes 3 and 1, a structural chromosomal abnormality between the two mentioned chromosomes [67] (p. 35); additional details about the structure of this derivative chromosomal abnormality were unavailable. The five patients with trisomy 8 were aged 39 to 85 years, with a median age of 60. All three patients with trisomy 8 as the only karyotypic abnormality were females, and both patients with additional clonal abnormalities were males. Bone marrow blast percentages were ≥10% < 20% in all five patients with trisomy 8.

The remaining abnormal karyotypes included the following: >3 abnormalities (a complex karyotype) in one Auer rod-negative patient, t(6;9)(p23;q34) in one Auer rod-positive patient, del(12)(p13) in one Auer rod-positive patient, and del(16)(q22) in one Auer rod-positive patient.

The IPSS scores (risk categories), available in 19 (86%) patients, were 0 (low risk) in 1 (4.5%), 0.5 (int-1) in three (14%), 1.0 (int-1) in three (14%), ≥1.5–2.0 (int-2) in nine (41%), and ≥2.5 (high) in three (14%) patients. The IPSS-R scores (risk categories) were 2.5 (low) in one (4.5%), 3.5–4.5 (int) in four (18%), >4.5–6.0 (high) in seven (32%), >6.5 (very high) in 3 (14%) patients, and at least >3.5–4.5 (int) in six (27%) patients with incompletely available data to calculate the scores.

Clinical follow-up to AML or death was available in 16 (73%) of 22 patients. Nine (41%) patients received a bone marrow transplant, including all three Auer rod-positive patients with <5% bone marrow blasts, three Auer rod-positive patients with 5% to <10% bone marrow blasts, two Auer rod-positive patients with 10% to <20% bone marrow blasts, and one patient in the group with ≥5% peripheral blood blasts. Seven of these nine patients were alive for 2 to 30 months post-transplant; all seven were Auer rod-positive. The remaining two patients developed post-transplant AML, including one Auer rod-positive patient with high IPSS-R risk at diagnosis who died of the disease. The other patient who developed post-transplant AML was in the ≥5% peripheral blood blast group with a very high IPSS-R risk score.

Four (18%) of 22 patients progressed to AML without receiving a transplant, including the index Auer rod-positive patient who developed AML unexpectedly pre-transplant, two other Auer rod-positive patients, including the patient in the ≥5% peripheral blood blast group, and the fourth patient also in the group with ≥5% peripheral blood blasts. Two of these four patients died, one Auer rod-positive and the other Auer rod-negative.

The remaining three patients with available follow-up died without developing acute leukemia, including one Auer rod-positive and two Auer rod-negative patients.

3.1.2. Statistical Tests for Determining Any Association of Hematologic and Clinical Parameters Between Auer Rod-Positive and Auer Rod-Negative Patients in All Patients with <20% Bone Marrow Blasts, *N* = 22

This section provides details of the statistical analyses for clarity. Tables 3 and 4 show the distribution of patients used for the statistical analyses of the development of acute leukemia and referral for a bone marrow transplant, respectively. Table 5 summarizes the results of the statistical analyses in this study.

a. Hemoglobin in Auer rod-positive (available values, $n = 12$) and Auer rod-negative patients (available values, $n = 6$): The U-value is 35.5. The critical value of U at $p < 0.05$ is 14. Therefore, the result is not significant at $p < 0.05$. The z-score is 0. The p-value is 1. The result is not significant at $p < 0.05$.

b. WBC counts in Auer rod-positive (available values, $n = 11$) and Auer rod-negative patients (available val-

Table 3: The distribution of patients for the statistical analyses for the development of acute leukemia.

N Patients	Positive for the Development of Acute Leukemia	Negative for the Development of Acute Leukemia [a]	Lost Follow-Up	Row Totals N
Auer rod-positive	4 [b]	8	3	15
Auer rod-negative	2	2	3	7
Column Totals N	6	10	6	22

[a] The numbers in this column include the three deaths described in the previous section's results; [b] Includes one patient from the group with ≥5% PB blasts.

Table 4: The distribution of patients for the statistical analyses for being referred and receiving a bone marrow transplant.

N Patients	Referred for and Received a BMT	Not Referred for a BMT	Lost Follow-Up	Row Totals N
Auer rod-positive	8	2	3	13
>5% peripheral blood blasts	1	5	3	9
Column Totals N	9	7	6	22

Abbreviation: BMT, bone marrow transplant.

ues, $n = 6$): The U-value is 31.5. The critical value of U at $p < 0.05$ is 13. Therefore, the result is not significant at $p < 0.05$. The z-score is −0.1005. The p-value is 0.92034. The result is not significant at $p < 0.05$.

c. Platelet counts in Auer rod-positive (available values, $n = 10$) and Auer rod-negative patients (available values, $n = 8$): The U-value is 30. The critical value of U at $p < 0.05$ is 17. Therefore, the result is not significant at $p < 0.05$. The z-score is −0.8441. The p-value is 0.4009. The result is not significant at $p < 0.05$.

e. The development of acute leukemia in Auer rod-positive and Auer rod-negative patients:

A chi-square test of independence in the 16 patients with available follow-up showed no association of Auer rods with the development of acute leukemia, X^2 (2, $N = 16$) = 0.3556, $p = 0.550985$. The Fisher exact test statistic value was 0.6044, which is not significant at $p < 0.05$.

f. Referral for receiving a bone marrow transplant in the two groups of RAEB-T patients diagnosed only due to Auer rods ($n = 13$) and ≥5% peripheral blood blasts:

Including only the 16 patents with available follow-up, the Fisher exact test statistic value was 0.035, which is significant at $p < 0.05$. The Fisher exact test is considered more reliable than the chi-square test in small samples [69]. However, a χ^2 test was also performed and showed the chi-square statistic was 6.1122, with $p = 0.013425$, which was also significant at $p < 0.05$.

Adding the six patients with lost follow-up as not having received a BMT, including three patients in each of the two diagnosis groups, to the 16 patients with available follow-up, the Fisher exact test statistic value was 0.0306, which is significant at $p < 0.05$. Similarly, the chi-square statistic was 5.5944, $p = 0.018018$, which was also significant at $p < 0.05$.

3.1.3. RAEB-T Patients with < 20% Bone Marrow Blasts and Auer Rods as the Only Diagnostic Criterion, *N* = 13

All three blood counts were available in 9 (69%) patients; one or two of the three counts were available in two (15%). Two patients with unavailable counts had transfusion- dependent anemia, with one of these also platelet transfusion-dependent. Hemoglobin values were >10 g/dL in three (23%), 8–10 g/dL in four (31%), <8.0 g/dL in three (23%), and unavailable in three (23%) patients with anemia, including two with transfusion-dependent anemia. WBC counts were 1.5–8.3 (median 1.8) × 10^9/L in nine (69%), with ANC 0.6–1.0 × 10^9/L in four (31%), and <0.8 × 10^9/L in one (7%). Platelets were 10–201 (median 84) × 10^9/L in ten (77%), ≥100 × 10^9/L in four (31%), 50–100 × 10^9/L in two (15%), <50 × 10^9/L in four (31%), and unavailable in three (23%), with one of these three platelet transfusion-dependent.

Bone marrow blast percentages ranged from 2.6%–15.0% (median 8.4%), with three patients having <5% (median 3.2%, range 2.6–4.0%) bone marrow blasts. Results of cytogenetic analyses were available in 12 (92%) patients and showed a normal karyotype in the majority

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Table 5: Association of patient age, gender, hematologic, and clinical parameters with Auer rod-positive or Auer rod-negative disease.

Variables Examined for any Association with Auer Rod-Positive Disease	*P* Values [a]
Male or female sex [b]	$p = 0.665$
Patient age at diagnosis [b]	$\mathbf{p = 0.019}$
Hemoglobin at diagnosis [b]	$p = 1$
WBC count at diagnosis [b]	$p = 0.920$
Platelet count at diagnosis [b]	$p = 0.400$
Development of acute leukemia [b]	$p = 0.889$
Referral for and receiving a bone marrow transplant [c]	$\mathbf{p = 0.013}$

[a] Significant *p* values are shown in bold text; [b] These analyses compared all Auer rod-positive patients with all Auer rod-negative patients; [c] This analysis compared the diagnosis of RAEB-T (or MDS-IB2) due to only the presence of Auer rods ($n = 13$) with the group having ≥5% peripheral blood blasts ($n = 9$).

(58%), including all (100%) patients with Auer rods and <5% bone marrow blasts. Five (42%) patients showed clonal karyotypic abnormalities, all classified as intermediate by the IPSS and good ($n = 1$) or intermediate ($n = 4$) by the IPSS-R cytogenetic subgroups. Notably, complex or poor prognostic cytogenetic abnormalities were absent in this group.

The IPSS scores (risk categories) were 0 (low) in one (8%) patient, 0.5 (int-1) in two (16%), 1.0 (int-1) in three (25%), 1.5 (int-2) in three (25%), 2.0 (int-2) in one (8%), 2.5 (high) in two (16%), and unavailable in one. The IPSS-R scores (risk categories) were 2.5 (low) in one patient (11%), 3.5 (int) in two (22%), ≥4.0 <4.5 (int) in one (11%), ≥ 5–6.0 (high) in four (44%), and 6.5 (very high) in one (11%). In four patients with incompletely available data, the IPSS-R scores were at least >3.5 (int or high) in one, >4.0 (int or high) in two, and >4.5 (probably high) in one.

Clinical follow-up to AML or death was available in ten (77%) patients, seven (70%) of whom received a BMT. Three (30%) patients developed AML, including one pre- and one post-transplant, and three (30%) died, including two post-AML. Of the remaining three patients, one with intermediate risk IPSS-R was alive at the last follow-up, and two were lost to follow-up as per the medical records {1 with unavailable blood counts and > 4.0 (int or high) IPSS-R, and 1 with unavailable cytogenetics and >3.5 (int) IPSS-R}.

3.1.4. RAEB-T Patients with < 20% Bone Marrow Blasts and ≥ 5% Peripheral Blood Blasts with or without Auer Rods, *N* = 9

All three blood counts were available in 8 (89%) patients; one had unavailable counts. All patients had peripheral blood cytopenias. Hemoglobin values were ≥10 g/dL in two (22%), 8–10 g/dL in 4 (44%), <8 g/dL in 2 (22%), and unavailable in one (11%). The WBC counts were 1.1–20.9 (median 4.7) × 10^9/L, with ANC 0.2–2.1 (median 0.9) × 10^9/L in five (56%). Platelet counts ranged from <15–266 (median 84.5) × 10^9/L in eight (89%), ≥100 × 10^9/L in four (44%), 50–100 × 10^9/L in three (33%), and <50 × 10^9/L in three (33%) patients.

Bone marrow blast percentages ranged from 5.4% to 15.0% (median 10%). Results of cytogenetic (karyotypic) analyses were available in 6 (67%) patients, including two normal and four (44%) abnormal karyotypes, including a complex karyotype with >3 clonal abnormalities in one. Trisomy 8 was the most common cytogenetic abnormality in the other three patients. As described in the section with all 22 patients, trisomy 8 was the only clonal karyotypic abnormality in two patients, and it was present with one additional abnormality (a derivative of chromosomes 3 and 1) in one patient. Notably, both Auer rod-positive patients in this group did not show complex or ≥3 cytogenetic abnormalities.

The IPSS scores (risk categories) were at least int-2 or high in six (86%) of seven patients with available values and int-1 in one (14%). The corresponding IPSS-R scores (risk categories) were high or very high in five (73%) of six, indicating an ominous prognosis for most patients in this group. Only one patient had a lower 0.5 (int-1) IPSS and Int-risk IPSS-R; of note, this patient was also Auer rod-positive.

Clinical follow-up, including progression to AML or death, was available for five patients. Three patients developed AML, including one post-BMT, and there were three deaths, including one post-AML. Of the other four patients, one received chemotherapy with no remission, one had high-risk IPSS-R, and two with unavailable cytogenetics/ IPSS-R received supportive therapy.

3.1.5. IPSS and IPSS-R Scores and Risk Categories Correlated with Clinical Outcomes

Table 6 shows the IPSS and IPSS-R scores, risk categories, and the available follow-up to AML or death for all study patients (n = 22).

All (100%) of the patients in this study with low or int-1 IPSS were Auer rod-positive. Of note, only one (11%) of the nine patients in the ≥5% peripheral blood blast group had an int-1 IPSS score; this patient was also Auer rod-positive and had intermediate-risk IPSS-R. All eight remaining patients with ≥5% peripheral blood blasts had at least int-2 or high IPSS and high or very high IPSS-R scores, predicting a serious prognosis.

3.2. Patients Classified According to the Fifth Edition of the WHO Classification in 2022

In Table 1, which is shown in Section 3.1. above, all 22 patients represent *de novo* MDS according to the WHO classification. One of these patients with t(6;9)(p23;q34) in the group with Auer rods as the only criterion was excluded since that patient would now be classified as AML with *DEK::NUP214* fusion by the WHO 2022 classification [34,70,71]. In the remaining 21 patients, classified as MDS-IB2 by the WHO 2022 classification, the adverse prognostic significance of 10–19% blasts in the bone marrow is well-established by prognostic systems for MDS, including the IPSS and IPSS-R [10,11]. Therefore, the nine patients in Table 1 with ≥10 to 19% blasts in the bone marrow were excluded to focus on the patients with only Auer rods or ≥5% peripheral blood blasts as the diagnostic criteria for MDS-IB2. Those remaining 12 patients are shown in Table 7, and the patient ages, male-to-female ratios, and the IPSS and IPSS-R prognostic risk scores for the MDS-IB2 sub-groups are shown in Table 8.

RAEB-T Patients with Less Than 5% Bone Marrow Blasts and Auer Rods Classified as MDS-IB2 by the WHO 2022 Classification, *N* = 3

The index patient showed Auer rods and <5% bone marrow blasts in two biopsies performed one year apart. This patient was referred to the University of Michigan for persistent neutropenia following granulocyte colony-stimulating factor (G-CSF) therapy. The bone marrow biopsy performed eleven months before referral with a presenting absolute neutrophil count of 0.6×10^9/L showed cellular marrow smears with 60% erythroid precursors, including occasional dysmorphic forms, and 2.6% blasts (1000-cell count) with a single, slender Auer rod in two blasts in the bone marrow, in the absence of granulocytic or megakaryocytic dysplasia or circulating blasts. A repeat bone marrow biopsy performed for cytogenetic analysis showed a normal karyotype with 50% marrow cellularity

Table 6: The International Prognostic Scoring System (IPSS) risk categories correlated with the Revised IPSS and follow-up to acute leukemia or death for both subgroups (N = 22).

IPSS Score, Risk Category	N with Available IPSS	N, IPSS-R Risk Category	% Auer Rod Group or ≥5% PB Blast Group	N with Available Follow-Up	N with Outcome AL or Death/N with Available Follow-Up
0, Low	1	1, Low	100% Auer rod	1	1/1 AL
0.5, Int-1	3	3, Int	100% Auer rod	3	1/3 AL
1.0, Int-1	3	1, High; 1 ≥ Int; 1, Int	100% Auer rod	2	1/2 Death
1.5–2.0, Int-2	9	1, Very high; 5, High; 2, Int or High; 1, >Int	67% ≥ 5% PB blast33% Auer rod	6	4/6 AL/death (1 AL post-BMT,1 death, 2 AL, death)
≥2.5, High	3	2, Very high1, High	67% ≥ 5% PB blast33% Auer rod	2	1/2 post-BMT AL and DOD
Total	19	19, Low to Very high IPSS-R	63% Auer rod37% ≥ 5% PB blast	14	8 [a]/14

Abbreviations: IPSS, International Prognostic Scoring System [10]; IPSS-R, Revised IPSS [11]; N, numbers of patients; PB, peripheral blood; AL, acute leukemia; LFU, lost follow-up; BMT, bone marrow transplant; DOD, died of disease. [a] One additional death with unavailable IPSS

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Table 7: Distribution of 12 patients having < 10% bone marrow blasts diagnosed as MDS-IB2 due to Auer rods alone or ≥ 5% peripheral blood blasts by the WHO 2022 classification [33,34].

Bone Marrow Blasts %	Numbers (%) of MDS-IB2 Patients with < 10% Bone Marrow Blasts According to the Fifth Edition of the WHO Classification		
	Auer Rods as the Only Criterion	≥5% Blasts in Peripheral Blood	
		Auer Rods Negative	Auer Rods Positive
Less than 5%	3 [a] (25%)	0 (0%)	0 (0%)
5 to <10 %	5 (42%)	3 (25%)	1 (8%)
Less than 10%	**8 (67%)**	**3 (25%)**	**1 (8%)**

Abbreviations: MDS-IB2, myelodysplastic syndromes with increased blasts-2; WHO, World Health Organization. The numbers in bold font show the total number (%) of patients in each group with less than 10% bone marrow blasts.

Table 8: Patient demographics, prognostic scores, and risk categories in 12 patients having < 10% bone marrow blasts diagnosed as MDS-IB2 due to Auer rods alone or ≥ 5% peripheral blood blasts by the WHO 2022 criteria.

	MDS-IB2 Patients with <10% BM Blasts, All N (%)	Auer Rods as the Only Defining Criterion			≥ 5% PB Blasts with or without Auer Rods		
		<5% BM Blasts	5–<10% BM Blasts	<10% BM Blasts	Auer Rods Negative	Auer Rods +	Auer Rods ±
Patients N	12	3	5	8	3	1	4
Age, median (range) years	59 (20–79)	44 (20–58)	61 (48–79)	59 (20–79)	62 (50–85)	34	58 (34–72)
Male: Female	8:4	2:1	4:1	6:2	2:1	0:1	2:2
IPSS scores/risk category	Available in 9 (75%)/12 patients	0/Low; 0.5/Int-1; 0.5/Int-1	1.0/Int-1; 1.0/Int-1; 1.0/Int-1; 1.5/Int-2; NA	Low/Int-1 (n = 6); Int-2 (n = 1)	2/Int-2; NA; NA	0.5/Int-1	Int-2 and Int-1, 2 NA
IPSS-R scores/risk categories	Low (n = 1); int (n = 4); high/int-to-high (n = 5); very high (n = 1)	2.5/Low; 3.5/Int; ≥4.0 [a] < 4.5/Int	5/High; >4/Int-to-high [b]; 3.5/Int; 5.5/high; >3.5/Int to high [c]	Low (n = 1); int (n = 3); high/ int-to-high (n = 4)	≥8/Very high [a]; >3.5/int-to-high [d]; NA [e]	≥4 [a] <4.5/Int	Very high; int-to-high; NA; and int

Abbreviations: MDS-IB2, Myelodysplastic syndromes with increased blasts-2; BM, Bone marrow; PB, peripheral blood; N, total numbers of patients; NA, not available; ANC, absolute neutrophil count; [a] ANC NA in one; [b] at least >4 IPSS-R score; severity of anemia and ANC NA; [c] cytogenetics, ANC, and platelet counts NA in the patient with IPSS NA; [d] cytogenetics and ANC NA in one; [e] cytogenetics and counts NA.

comprised of 75% erythroid precursors showing megaloblastoid change and occasional dysmorphic forms, no ringed sideroblasts, barely 5% blasts (1000-cell count), no Auer rods, rare hypolobated neutrophils, and no circulating blasts. Three weeks after the second biopsy, a third bone marrow biopsy showed rare Auer rods in the bone marrow, which now had <1% blasts, 77.8% erythroid precursors, and, again, a normal karyotype. Peripherally, now there was severe neutropenia (hemoglobin 12.4 g/dL, ANC 0.3 × 10^9/L, WBC 1.2 × 10^9/L, platelets 143 × 10^9/L) and no circulating blasts. The patient was given G-CSF therapy and, at pre-BMT evaluation after two months, showed, unexpectedly, a packed bone marrow diagnostic of AML, with a normal karyotype and with Auer rods. The patient received a bone marrow transplant after complete remission following chemotherapy.

The second patient presented with anemia (hemoglobin 8.9 g/dL) and thrombocytopenia (platelet count 73 × 10^9/L), with normal WBC and ANC. A bone marrow biopsy showed 95% cellular marrow comprised of 75% erythroid precursors showing megaloblastoid change and nuclear dysmorphism (in 1 in 10 cells), 3.2% blasts (4% myeloblasts by flow cytometry) with rare Auer rods in the absence of granulocytic or megakaryocytic dysplasia, and no clonal cytogenetic abnormality. The patient received a bone marrow transplant, and a bone marrow biopsy, including cytogenetics, was normal 245 days after the transplant.

The third patient was referred for persistent pancytopenia after receiving chemotherapy at an outside facility following a bone marrow biopsy performed at the referring institution due to initially presenting with the fol-

lowing laboratory values: hemoglobin 7.2 g/dL, WBC 1.8 $\times$ 10^9/L, and platelets 10 $\times$ 10^9/L. A review of this previous biopsy performed at the time of referral to the University of Michigan showed 90% cellular marrow, 60% erythroid precursors, including rare, multinucleated dysmorphic forms, 5.3% marrow blasts, no Auer rods, and a normal karyotype. Another bone marrow biopsy was performed at referral 13 months after the first biopsy; this biopsy showed occasional Auer rods in 4% bone marrow blasts, dysmorphic megakaryocytes and erythroid precursors, and no circulating blasts. The patient underwent a bone marrow transplant, and six months post-transplant, there was no evidence of disease.

4. Discussion

The study findings are discussed similarly to the organization of the results, first comparing with earlier RAEB-T studies and subsequently in context with recent studies. Table 9 shows a comparison of the features of the patients in this study with earlier studies for RAEB-T patients with <20% bone marrow blasts diagnosed as RAEB-T due to the presence of Auer rods or ≥5% peripheral blood blasts.

As shown in Table 9, the median ages in this study were similar to those in previous studies [64,72,74]. Michels et al. studied 52 patients with RAEB-T, including patients with 20–30% blasts [73]. Auer rods were identified in blasts in all RAEB-T patients (n = 20) with ages less than or equal to 45 years and in 18 (56%) of 32 patients older than 45 years in their study [73] (p. 2341). However, the numbers of patients in the three categories of RAEB-T, including the two groups with less than 20% bone marrow blasts, are unavailable from the report [73]. In contrast, the current study showed that patients with Auer rod-positive MDS and less than 20% bone marrow blasts were younger than the patients with ≥5% peripheral blood blasts and <20% bone marrow blasts, and this difference was statistically significant. Interestingly, a significantly younger median age of 55 years was reported in the patients diagnosed only due to the presence of Auer rods in the study by Strupp et al.; however, the authors stated that they could not explain the significance of the difference in ages in their Auer rod-positive group [64] (p. 403).

Further, males predominated in the Auer rod-positive group in this study, as observed in the other large US study and two other studies [72,74,75]. Still, this study cohort was in contrast with the Strupp et al. study, which had a predominance of females [64].

In our Auer rod group, the median bone marrow blast percentage included three patients with < 5% bone marrow blasts, which explains the lower median bone marrow blast percentage than the other studies shown in Table 9. Notably, karyotypic findings were normal in 58% of the Auer rod group, like the study by Seymour et al. [72]. There were no (0%) complex karyotypes in the Auer rod-positive patients in this study, similar to fewer (24%) abnormal karyotypes in the same group by Strupp et al., with only 6% being complex karyotypes in their study [64]. Trisomy 8 was this study's most common cytogenetic abnormality, present in five (55.5%) of nine patients with abnormal karyotypes. This finding is consistent with trisomy 8 being the most frequent numerical aberration in MDS [76], the second most frequent abnormality in MDS after monosomy 7 [77], and the most frequent sole cytogenetic aberration in MDS [77].

In contrast, among the 110 patients with reported karyotypes in the study by Strupp et al. [64], trisomy 8 was only present in the patients with >20% bone marrow blasts, which were excluded in this study. None of the patients (n = 0) in the <20% bone marrow blast subgroups were reported to have trisomy 8 [64] (p. 399). The absence of trisomy 8 in the cases reported by Strupp et al. is explained here. Firstly, cytogenetic results were reported in only 35% (n = 110/310) of patients in the Strupp et al. study [64], in contrast to this study, wherein cytogenetic analyses were available for 82% (n = 18/22) of patients. As stated by the authors, "the IPSS was not evaluated because too many cytogenetic data were missing [64] (p. 401)." Secondly, among their 110 patients with reported cytogenetics results, the numbers of patients in each of the three subgroups of RAEB-T are not stated in the publication [64] (p. 399). Deriving the numbers from the percentages, since 1% of all cases in the group with 20–30% bone marrow blasts and in the entire group (n = 110) are reported for another (5q-) cytogenetic abnormality in Table 3 in their publication [64] (p. 399), it seems that 100 of 110 patients were included in the 20–30% bone marrow blast group. If that is indeed true, then both of their groups with less than 20% bone marrow blasts included only ten patients with available cytogenetics, comprising only 15% of the total 68 patients in their two groups compared with 82% (n = 18) of patients in the same two groups in this study. Thirdly, trisomy 8 has been reported in 19% of RAEB-T patients and 9.1% of RAEB-T patients as a sole abnormality [77]. The frequency of trisomy 8 as a sole cytogenetic abnormality in MDS has been reported as 9.3% in North America and 12% in Europe [77]. Therefore, the difference in the percentages of cases evaluated for cytogenetics, as calculated above between this study and the Strupp et al. study, and the very low percentage of cases assessed in these two groups by Strupp et al. is the most likely explanation for the lack of trisomy 8 in both of their two groups of RAEB-T with less than 20% bone marrow blasts.

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Table 9: Patient characteristics in this study compared with earlier studies with identifiable cases having < 20% bone marrow blasts and Auer rods.

RAEB-T patients with < 20% bone marrow blasts, studied characteristics	This study	Strupp et al. 2003 [64]	Seymour et al. 1993 [72]	Michels et al. 1989 [73]	Seigneurian et al. 1982 [74]	Weisdorf et al. 1981 [75]
Study cohort institutional source, country	5-y single institution, USA	30-y Registry, Germany	20-y single institution, USA	16-y single institution, USA	5-y single institution, France	Single institution, USA
Total N (%) < 20% bone marrow blasts	22 (100)	68 (100)	NA [a]	NA [a]	Not studied	Not studied
N (%) ≥5% peripheral blood blasts, <20% bone marrow blasts, ± Auer rods	9 (41%)	32 (47%)	NA [a]	NA [a]	Not studied	Not studied
Age median, years	62	65				
Age range, years	34–85	21–88				
% Males	44	88				
% bone marrow blasts, median	10	13				
Karyotype abnormal	67%	60%[b]				
Karyotype >3 abnormalities (% of abnormal)	25%	45%[b]				
N, ≥5% peripheral blood blasts and <5% bone marrow blasts ± Auer rods	0	0	NA [a]	NA [a]	Not studied	Not studied
N, < 20% bone marrow blasts + Auer rods	13 (59%)	36 (53%)	23 [c]	23	6	5
Age median, years	59	55	61 [d]	NA [a]	**51**	**73**
Age range, years	20–79	21–87	33–77		14–79	61–84
% Males	77	39	74	NA [a]	66	100
% bone marrow blast, median	8.4	12	12 [d]	NA [a]	12	9
Karyotype						
Normal	58%	76% [b]	56.5%	NA [a]	Not studied	Not studied
>3 abnormalities	0%	6% [b]	NA		Not studied	Not studied
N, <5% bone marrow blasts + Auer rods	3 (12%)	0 (0)	4	NA [a]	0	0
N, 5%–< 20% bone marrow blasts + Auer rods	8 (33%)	36 (53%)	19	NA [a]	6	5

Abbreviations: RAEB-T, refractory anemia with excess blasts in transformation; NA, not available; [a] Number unavailable from the report; [b] See the text in the discussion; [c] Number excluding chronic myelomonocytic leukemia in transformation [72]; [d] For the 5–19% bone marrow blast group.

In our group with ≥5% peripheral blood blasts, although the median bone marrow blast count was slightly lower (10%) than in the Dusseldorf group (13%), the patients' IPSS-R scores also predicted an ominous outcome, similar to the serious prognosis in their group akin to that of AML. In addition, the prognoses in their group with ≥5% blasts were worse than that of their Auer rod group [64], similar to this study. No MDS patient in this study was found to have ≥5% peripheral blood blasts and <5% bone marrow blasts, like Strupp et al., as would be expected if peripheral blood blasts represent a direct function of bone marrow blasts. Further, the karyotypes in the patients with ≥5% peripheral blood blasts were abnormal more often (67%) than in the Auer rod group and included complex abnormalities. These findings are similar to and confirm those by Strupp et al., with 60% abnormal karyotypes in their ≥5% peripheral blood blast group that were often complex (45%), in contrast with fewer (24%) abnormal karyotypes in their Auer rod group. Ohyashiki et al. also reported complex karyotypes in four (80%) of five patients with RAEB-T having ≥5% peripheral blood blasts and a serious prognosis [78].

The collective findings in this study of 22 patients, including the above-described karyotypic findings, which are similar to those by Strupp et al. in both of these groups and similar to Seymour et al. for the Auer rod group, suggest that the Auer rod-positive group may be genetically and biologically different, and therefore, may have a different pathogenetic pathway from the group having ≥5% peripheral blood blasts. The younger age of the Auer rod-positive group was significantly different from the ages of the Auer rod-negative group in this study, and Auer rod-positive patients are more likely to be referred for a bone marrow transplant than the ≥5% peripheral blood blast group, indicating that Auer rod-positive MDS may also be clinically distinct. Further, this study suggests that particularly when bone marrow blast percentages are low, Auer rod-positive MDS may represent an earlier or less aggres-

sive disease compared with MDS having ≥5% peripheral blood blasts, which almost always have a serious adverse prognosis akin to AML.

Next, primary MDS patients with <5% bone marrow blasts and Auer rods appear to be very rare, with most previous extensive studies on MDS not reporting any RAEB-T patients with <5% bone marrow blasts and Auer rods. As shown in Table 5, no (0%) patient with <5% bone marrow blasts and Auer rods was identified in the 30-year study of the Dusseldorf Registry [64], and four patients were identified in the 20-year U.S. study by Seymour et al. [72]. In addition to these patients, Table 10 shows patient characteristics in two case reports with <5% bone marrow blasts and Auer rods [79,80] and seven selectively identified patients by the multi-institutional search that was performed as described in the introduction [38].

In their publication in 2005, Willis et al. reported nine patients, including two with CMML in transformation. Patients with that diagnosis were excluded from this study since CMML was excluded from the category of MDS by the WHO classification in 2001, as depicted earlier in Figure 1. For the remaining seven patients reported by Willis et al. [38], the IPSS scores reported in their publication were 1.0, int-1 (n = 4), zero, low (n = 2), and unavailable (n = 1) in one patient [38]. The calculated IPSS-R risk groups and other patient characteristics and outcomes for the seven patients included in the published report [38] are shown in Table 11.

As shown in Table 11, four patients reported by Willis et al. having MDS with <5% bone marrow blasts and Auer rods had a high-risk IPSS-R score, which indicates that there were clinical, hematologic, and genetic factors that accounted for the adverse prognostic risk in these patients; even without knowledge of the genetic abnormalities in these patients, the high-risk IPSS-R scores predict a serious prognosis. The only patient with an intermediate-risk IPSS-R score was given supportive care, and this patient died without progressing to AML, suggesting that the patient likely had other comorbidities that caused the poor outcome. The only patient with a low-risk IPSS-R score in their report was a patient with findings of refractory anemia with ringed sideroblasts, except for the presence of

Table 10: Patient characteristics in this study compared with earlier reports of identifiable RAEB-T patients having < 5% bone marrow blasts and Auer rods.

RAEB-T patients with < 20% bone marrow blasts, studied characteristics	This study	Willis et al. 2005 [38]	Wong et al. 2002 [79]	Bernardeschi et al. 1994 [80]	Seymour et al. 1993 [72]
Study cohort institutional source, country	5-y single institution, USA	Multiple institutions, USA	Single institution, China	Single institution, Italy	20-y single institution, USA
Identification of studied RAEB-T patients	Consecutive patients	Selectively identified	Case report	Case report	Consecutive patients
Total N, < 20% bone marrow blasts	22 (100%)	Not studied	Not studied	Not studied	NA from report
N, < 20% bone marrow blasts + Auer rods	13 (59%)	Not studied	Not studied	2 [a]	23
N (%), < 5% bone marrow blasts + Auer rods	3(12%)	7	1	1	4
Age median, years	44	64	32	58	68
Age range, years	20–58	8–75	NA	58	33–70
% Males	66%	57%	0	0	100%
% bone marrow blasts, median	3.2	3.8	normal	3.5	2
% bone marrow blasts, range	2.6–4.0	2–4.9	normal	3.5	2–4
Karyotype normal	100%	33%	0	NA	50%
Revised International Prognostic Scoring System (IPSS-R) risk category calculated from the publications	int (n = 2), low (n = 1)	≥int/high (n = 4); ≥low/int (n = 2); NA (n = 1) [a]	int	NA	NA [b]
N, 5%–<20% bone marrow blasts + Auer rods	8 (33%)	Not studied	Not studied	1	19

Abbreviations: RAEB-T, refractory anemia with excess blasts in transformation; N, number; NA, not available; [a] Cytogenetics unavailable; [b] IPSS int-1 in all four patients.

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Table 11: The calculated IPSS-R scores and patient characteristics in the 7 reported patients with MDS having <5% bone marrow blasts and Auer rods in the publication by Willis et al. [38].

N [a]	Age	Sex	IPSS-R Score	Other Patient Characteristics and Outcomes Reported in the Publication
1.	63	F	Int Risk	Patient given only supportive care; died at 7 months due to gastrointestinal bleeding and pneumonia without progression to AML
2.	8	M	High Risk	Patient progressed to AML in 6 months, died 3 months after BMT
3.	74	M	Low Risk	Diagnosis of refractory anemia with ringed sideroblasts if Auer rods were absent; patient progressed to AML in six months
4.	64	F	Unavailable cytogenetics	Hypocellular bone marrow (10% marrow cellularity) with absent iron stores; patient died at one month due to unknown cause without progression to AML
5.	55	M	High Risk	BMT followed by progression to AML and death
6.	66	M	High Risk	Death due to bone marrow failure at 4 months without progression to AML
7.	43	F	High Risk	Progression to AML at 5 months and death 7 months later due to AML relapse

Abbreviations: N, case number; F, female; M, male; IPSS-R, International Prognostic Scoring System, Revised [11]; AML, acute myeloid leukemia; BMT, bone marrow transplant; [a] The case numbers correspond to the case numbers in the publication [38] except for # 7 in the table, which was # 8 in the publication [38]; the two excluded cases of CMML in transformation were numbered 7 and 9 [38].

Auer rods; this patient could have harbored genetic mutations to explain the reported adverse outcome [48,81–83]. Lastly, the seventh patient, with unavailable cytogenetics, hypocellular bone marrow with 10% marrow cellularity, and absent iron stores, who died one month after the diagnosis due to an unknown cause, also suggests other causes for that patient's demise.

IPSS-R scores provide greater prognostic significance than the IPSS due to their consideration of the depth of cytopenias, further stratification of bone marrow blast percentages, and karyotypic abnormalities [11]. Therefore, higher IPSS-R scores in Auer rod-positive MDS with low bone marrow blast percentages indicate that variables other than Auer rods existed in such cases to account for the worse prognosis, and the presence of Auer rods alone does not predict a worse outcome.

Indeed, the patient reported by Wong et al. had no increase in bone marrow blasts, del(13)(q12q14) by cytogenetics, a calculated intermediate-risk IPSS-R, and no disease progression for several years despite the presence of Auer rods [79]. In contrast, the index patient described in this study who progressed to AML after the presenting neutropenia became progressively severe and showed temporal variability in the presence of Auer rods during disease progression. Nonetheless, since severe neutropenia significantly increases transformation to AML in low-risk MDS [84], and the presence of severe neutropenia in lower-risk MDS may itself be considered an indication for performing a BMT [85], the presence of Auer rods alone cannot be attributed as having an adverse prognostic impact in this study patient with severe neutropenia. Intriguingly, a rare case of spontaneous remission of Auer rod-positive RAEB-T with 25% myeloblasts in the bone marrow in a pregnant woman has been reported [86]. The patient refused treatment with chemotherapy and was well two weeks after the healthy baby's delivery, and a repeat bone marrow biopsy showed complete hematologic remission [86].

Therefore, given all the above results and critical analysis of previous studies, based on the strength of the IPSS-R, there is virtually no evidence for classifying MDS patients with less than <5% bone marrow blasts only due to the presence of Auer rods in the highest-risk RAEB-2 category. This classification, introduced in the WHO 2008 guidelines and continuing as a diagnostic criterion in the WHO 2022 classification, lacks supporting evidence. Accurate determination of prognostic risk is critical for each patient with MDS, regardless of the rarity of the subsets of patients. The WHO classification is meant to be used worldwide in countries with limited resources where reliance on morphologic evaluation is still critically important. As has been written by previous authors [19,66],

this study and critical analysis indicate that Auer rods should not be included as a sole diagnostic criterion for the highest-risk category of MDS.

Moreover, several studies have shown the favorable prognostic impact of the presence of Auer rods in the treatment of patients with Auer rod-positive MDS versus Auer rod-negative MDS. In the 1989 study by Michels et al., the survival of RAEB-T patients aged >45 years treated by induction chemotherapy was better in the Auer rod-positive patients than in Auer rod-negative patients [73]. Another study in 1989 showed a better treatment response in patients with Auer rod-positive RAEB-T with high percentages of bone marrow blasts (16%–29%) [87]. Seymour and Estey showed superior complete remission rates and survival in RAEB-T patients with Auer rods treated with intensive chemotherapy than Auer rod-negative patients [19,72]. A study in 2018 showed the favorable impact of the presence of Auer rods on achieving complete remission and extending survival in patients with high-risk MDS and secondary AML [88].

Further, two recent studies have studied patients with MDS having excess blasts-2 (MDS-EB2) and Auer rods in the context of molecular genetic abnormalities in these patients. First, Huang et al. studied 516 consecutive patients with MDS-EB2 diagnosed according to the WHO 2016 criteria, including 25 (~5%) patients with a median age of 40 years (range 16–84) diagnosed only due to the presence of Auer rods [62]. Those 25 patients included 68% males, consistent with the male dominance in the presented study, and 72% normal karyotypes, similar to this and other previous studies. However, 67% of their 25 Auer rod-positive patients had high or very high IPSS-R scores, and 28% had intermediate-risk IPSS-R, indicating that bone marrow blast percentages, which are unavailable from the publication, were likely to be high in these patients [62]. Their cohort also included 34 Auer rod-positive patients meeting the criteria for blast percentages in peripheral blood, bone marrow, or both specimens for MDS-EB2, notably with high or very high IPSS-R risk in 93% of those 34 cases [62]. Next-generation sequencing of bone marrow samples in 39 Auer rod-positive patients (with an unavailable distribution of patients from the above-described two Auer rod-positive cohorts) showed mutations most frequently in *NPM1*, followed by *DNMT3A*, *NRAS*, *TET2*, *U2AF1*, *WT1*, and *IDH1,* and the absence of *TP53* gene mutations [62]. In contrast, *U2AF1*, *TP53*, and *SF3B1* were most commonly mutated in Auer rod-negative patients (n = 85), which, as a group, included 25% of patients with a complex karyotype [62].

Subsequently, Wang et al. studied 112 adult patients with MDS-EB2, including 32 Auer rod-positive and 80 Auer rod-negative, who received a myeloablative allogeneic hematopoietic stem cell transplant at their center in China between 2015 and 2020. They found that Auer rods were significantly associated with better pre-transplant therapeutic responses and improved survival. [63]. The median age for their Auer rod-positive patients was 42 years (range 18–59 years), and 15 of their Auer rod-positive cases were diagnosed as MDS-EB2 only due to the presence of Auer rods. In contrast with this study with a predominance of males, their Auer rod-positive patients included 18 (56%) females and 14 (44%) males, which could be explained by selection bias and the fact that their cohort included only patients who underwent a transplant, as also stated by the authors [63]. Significantly, like the presented study, they also reported a predominance of a normal karyotype in 75% of their Auer rod-positive cases, with only a rare (3%) patient with an unfavorable karyotype. Bone marrow blast percentages were not given. However, the IPSS-R scores were reported to be high or very high in 90% of their 32 Auer rod-positive MDS-EB2 patients, indicating that bone marrow blast percentages were likely to be high in their patients [63]. They reported a distinct genetic mutational profile for their patients with Auer rods with *U2AF1, NRAS, DNMT3A, NPM1*, and *WT1* as the most frequently mutated genes in conjunction with the notable absence of mutations in *TP53* [63]. Mutations in *ASXL1* were present at a lower frequency (~4%) in the Auer rod-positive group [63]. Since mutations in *SRSF2, SF3B1, U2AF1, ZRSR2, ASXL1*, *EZH2*, *BCOR*, or *STAG2* genes indicate the presence of a secondary AML versus *de novo* AML [47], the presence of *U2AF1* and *ASXL1* mutations in their patients [63] and the cohort by Huang et al. [62] indicates that their cohorts likely included patients with both *de novo* and secondary MDS with high blast counts. In contrast with both of these recent studies, the presented study included only *de novo* MDS patients classifiable as MDS-EB2 or MDS-IB2.

Although beyond the scope of the current study, it would be valuable to further study these rare patients with Auer rod-positive MDS and low blast counts with molecular genetic correlation. Until then, patients with MDS, low blast counts, and Auer rods would be better served by a complete evaluation of the integrated clinical, pathologic, and genetic, including cytogenetic and molecular genetic findings on an individual basis for diagnostic classification, with risk stratification for clinical management based on the widely recognized and utilized prognostic scoring systems.

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5. Conclusions

(1) This study shows that MDS patients with Auer rods and <5% bone marrow blasts are extremely rare or maybe rarely recognized and underreported.
(2) Applying the widely used IPSS-R prognostic scoring system shows higher risks than the IPSS in most Auer rod-positive MDS patients with <5% bone marrow blasts, indicating that the IPSS-R variables, not Auer rods, account for the unfavorable prognosis. Each of these patients with low blast counts requires individual evaluation for all currently recognized risk factors, including those determined by molecular genetic analysis.
(3) In this small study, Auer rod-positive MDS patients with MDS-IB2 were significantly younger than MDS-IB2 patients with ≥5% peripheral blood blasts. Auer rod-positive MDS patients, as a group, were significantly associated with referral for a BMT in this study but not with the development of acute leukemia. *De novo* MDS with Auer rods diagnosed as MDS-IB2 only due to the presence of Auer rods, as a group, comprise patients with lower risks by IPSS-R, compared to the group diagnosed only due to the presence of ≥5% peripheral blood blasts, which almost always have high or very high-risk scores predicting an ominous outcome. MDS with Auer rods shows predominantly normal karyotypes with virtually none or very few complex karyotypes compared to the ≥5% peripheral blood blast group, which harbors primarily abnormal and, more often, complex karyotypes.
(4) This study suggests that Auer rod-positive *de novo* MDS, especially when bone marrow blasts are low, are likely to be clinically, genetically, and biologically distinct and different from MDS with ≥5% peripheral blood blasts, which almost always show an excess of bone marrow blasts and an ominous prognosis akin to AML.
(5) No rationale is identified in this study, including with a literature review, for including patients with MDS having <5% bone marrow blasts and Auer rods as the sole criterion for the highest-risk MDS category in the current WHO classification.
(6) Additional studies of patients with MDS and Auer rods are required in the context of other variables, including percentages of bone marrow blasts and molecular genetic analyses, to clarify the prognostic significance, if any, of the presence of Auer rods beyond their indication of a neoplastic process, especially in the presence of less than 5% bone marrow blasts. Auer rods are readily identifiable by a careful visual examination of smear preparations, which is required anyway for differential cell counts in bone marrow aspirate and peripheral blood smear preparations. For future studies, if an evaluation of the cytomorphologic elements of hematologic dysplasia may be desired to distinguish any other possible distinctive cellular morphologic features of the elements of hematologic dysplasia in the hematopoietic cells in Auer rod-positive and Auer rod-negative MDS, then comprehensive large studies comprising at least hundreds of patients with MDS are suggested using digital morphologic evaluation and artificial intelligence methods.

6. List of Abbreviations

AML	acute myeloid leukemia
ANC	absolute neutrophil count
BMT	bone marrow transplant
FAB	French American British
G-CSF	granulocyte colony-stimulating factor
IPSS	International Prognostic Scoring System
IPSS-R	International Prognostic Scoring System, Revised
MDS	myelodysplastic neoplasms, or myelodysplastic syndromes
MDS-IB2	Myelodysplastic neoplasms with increased blasts-2
RAEB	Refractory anemia with excess of blasts
RAEB-2	Refractory anemia with excess of blasts-2
RAEB-T	Refractory anemia with excess of blasts in transformation
WBC	white blood cell
WHO	World Health Organization

Author Contributions

The author confirms that she was solely responsible for the conception, design, analysis, interpretation, drafting, and final approval of the article.

Availability of Data and Materials

All data generated or analyzed during this study are included in this published article.

Human Rights Statement

The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board for Human Subject Research (IRBMED) of the University of Michigan, Ann Arbor, Michigan, USA.

Consent for Publication

Not applicable.

Conflict of Interest

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