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MYELODYSPLASTIC SYNDROMES (M SAVONA, SECTION EDITOR)

The Clinical Challenge of Idiopathic Cytopenias of Undetermined Significance (ICUS) and Clonal Cytopenias of Undetermined Significance (CCUS)

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Abstract

Purpose of Review To review terminology, diagnostic algorithms, and clinical outcomes for patients with unexplained blood cytopenias.

Recent Findings Patients with cytopenias that remain unexplained after organized diagnostic testing can be described as having "idiopathic cytopenias of undetermined significance" (ICUS). Patients with unexplained cytopenias have a risk of progression to clonal myeloid neoplasms, including myelodysplastic syndromes (MDS). If a somatic mutation in a gene associated with leukemia is detectable in hematopoietic tissue, especially if multiple mutations with a high (> 10%) variant allele frequency are present, the risk of progression to frank neoplasia is greater than if such mutations are not detected. These patients can be described with the term, "clonal cytopenias of undetermined significance" (CCUS). CCUS patients have a natural history similar to lower-risk MDS.

Summary For patients with unexplained cytopenias, longitudinal follow-up including serial monitoring of blood counts is appropriate in view of the progression risk. Genetic testing may aid in risk stratification.

Keywords Cytopenias · Next-generation sequencing · Myelodysplastic syndromes · Clonal hematopoiesis · Ineffective hematopoiesis · Diagnostic testing

Introduction

Hematologists are often asked to evaluate patients with blood count abnormalities that include cytopenias [1]. Sometimes, despite a careful and organized assessment of common etiologies for blood cytopenias such as nutritional deficiency, hematologic neoplasia, myelosuppressive medication, or an autoimmune disorder, a patients' abnormal laboratory results remain unexplained [2]. This challenging situation is all too common and can be frustrating for patients and unsatisfying for clinicians.

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While many patients with unexplained cytopenias have stable blood counts for years and do not experience an alteration in either life expectancy or quality of life as a result of the cytopenias, some patients do suffer clinical consequences, and progression to an overt hematologic neoplasm such as myelodysplastic syndromes (MDS) can also occur. In addition, at the population level, even mild cytopenias appear to be associated with an increase in morbidity and all-cause mortality. This association between blood count and increased morbidity and mortality is best studied for anemia in older persons, but is also true of neutropenia and thrombocytopenia [3, 4, 5•, 6–8].

The advent of routine genetic sequencing in clinical practice can help bring clarity to the etiology of unexplained cytopenias in some patients. In recent years, investigators have come to understand that patients with cytopenias and certain somatic DNA mutations that can be detected in blood or marrow are at increased risk of subsequent progression to overt neoplasia, and detecting these mutations may influence recommended surveillance [9••].

This review summarizes some of the terminology that is being used to describe patients with unexplained cytopenias,

as well as surveying emerging tools for risk stratification and prediction of clinical outcomes in such patients.

Definitions

Terminology used to describe patients with unexplained cytopenias and with clonally restricted hematopoiesis can be confusing and is evolving (Table 1) [13, 14]. Shortly before DNA sequencing became a routine part of clinical practice, cytopenic patients in whom extensive hematological evaluation had been undertaken but no specific diagnosis reached began to be described using the term "idiopathic cytopenias of undetermined significance" (ICUS)—a term formally defined in 2007, and a successor to vague expressions such as "not yet MDS" or "not quite MDS" that were used informally in the 1980s and 1990s in recognition of both the danger of MDS and difficulty in diagnosing the disease in some cases [15]. Several groups have since published guidance papers to help clinicians navigate an appropriate evaluation for patients with unexplained cytopenias (Table 2) [12••]. In brief, recommended evaluation includes clinical assessment or tests for nutritional deficiencies (specifically vitamin B_{12}, folate, iron, and copper), infections such as human immunodeficiency virus or hepatitis, renal failure or chronic inflammation, drug and toxin exposure (including ethanol and occupational toxins as well as prescription medications), neoplasia (especially MDS and other myeloid neoplasms), hypersplenism, and autoimmunity.

Since 2009, more than 50 different recurrent gene mutations associated with MDS and other myeloid neoplasms have been described [16]. Initially, it seemed that testing for the presence of these mutations would allow clinicians to diagnose MDS or other clonal myeloid neoplasms without difficulty, in the same way that the advent of clinical JAK2 mutational testing in 2005 helped provide diagnostic clarity for a large number of patients with unexplained erythrocytosis for whom polycythemia vera was a possibility, but full diagnostic criteria for that myeloproliferative neoplasm were not satisfied [17]. However, in 2014, several investigational groups described a high frequency of somatic mutations in leukemia-associated genes in the blood in older individuals in the absence of hematologic malignancy as defined by World Health Organization (WHO) diagnostic criteria [18–20]. This biological state (not a disease) is, termed clonal hematopoiesis of indeterminate potential (CHIP, defined as requiring a specific variant allele frequency (VAF) of > 2%) or age-related clonal hematopoiesis (ARCH, which does not require a specific VAF), is exceptionally common with aging, and confounds simple diagnostic link between somatic mutation and diagnosis of malignancy [10, 11, 21]. Instead of adding diagnostic clarity, clinicians who receive information about low-VAF mutations in common CHIP-associated genes such as *DNMT3A*, *TET2*, or *ASXL1* are often left wondering whether the detected mutation explains the patient's cytopenias or is merely an additional incidental finding not contributing to marrow failure [22].

We do not have universal agreement on minimal diagnostic criteria for MDS, and assessment of morphologic dysplasia is to some extent subjective, so there can be disagreement between clinicians on whether a patient meets criteria for MDS or not [23–25]. Experienced morphologists may disagree about whether enough dysplasia is present in a specific case to be confident about the diagnosis of MDS; the WHO minimal criteria of 10% dysplastic cells in a given lineage are

Table 1 Definitions of cytopenic states and related conditions

Abbreviation	Term	Considerations
CH	Clonal hematopoiesis	All hematopoiesis is ultimately clonally derived; the CH term is a shorthand that is usually used to refer to hematopoiesis that is imbalanced or marked by acquired mutation(s).
CHIP	Clonal hematopoiesis of indeterminate potential	Defined as somatic mutation in leukemia-associated "driver" gene at VAF ≥ 2% in the absence of a WHO-defined malignancy (Steensma et al. [10])
ARCH	Aging-related clonal hematopoiesis	Informal term for a hematopoietic clone, usually marked by a somatic mutation, with an outsized contribution to hematopoiesis that occurs in older persons, no specific VAF. Almost universal with aging (Shlush [11])
ICUS	Idiopathic cytopenia(s) of undetermined significance	Term used to describe a patient with unexplained cytopenia(s) despite careful evaluation (see Table 2) that is not known to have a clonal mutation in a putative leukemia driver gene, either because testing was not performed or because it was performed and no mutation was detected (Valent et al. [12••]).
CCUS	Clonal cytopenia(s) of undetermined significance	This refers to a patient with WHO-defined cytopenia who also has mutation in a leukemia-associated gene; progression risk relates to VAF and number and type of mutations, with combinations including splicing mutations conferring special risk (Malcovati et al. [9••]). Natural history similar to lower-risk MDS

WHO, World Health Organization; *VAF*, variant allele frequency

Table 2 Diagnostic evaluation of patients with unexplained cytopenias

Minimal diagnostic evaluation in cases of unexplained cytopenias:
History and physical examination focused on the presence of splenomegaly or other signs that might explain cytopenias, possible drug or toxin exposures, and physical dysmorphology (e.g., radial ray defects, skin pigmentation abnormalities).
Complete blood count with leukocyte differential
Peripheral blood smear
Serum ferritin level
Serum B_{12} level, with methylmalonic acid assessment if B_{12} level is < 400 pg/mL
Red blood cell folate; serum homocysteine measurement may supplement this.
Serum creatinine and C-reactive protein
Bone marrow aspirate and trephine (core) biopsy with cytochemistry and immunohistochemistry
Bone marrow flow cytometry, if local expertise is available
Bone marrow karyotype
If karyotyping fails, a fluorescent in situ hybridization (FISH) panel focused on the most common MDS-associated anomalies (chromosomes 5, 7, 8, 20, Y); these tests are likely not necessary if 20 metaphases are successfully karyotyped.
Next-generation sequencing including mutations commonly associated with myeloid neoplasms, if such testing available
Supplemental tests useful in special circumstances
If the patient is younger than age 40, with an abnormal karyotype but without a history of exposure to ionizing radiation or a DNA-damaging chemical (therapeutic or accidental, perform chromosome breakage analysis (i.e., karyotyping after exposure to a clastogenic agent such as mitomycin C or diepoxybutane)
If the patient has short stature or radial ray anomaly, chromosome breakage analysis to rule out Fanconi anemia
If the patient has abnormal fingernails or toenails, or oral leukoplakia or another abnormality of the tongue or buccal mucosa, perform telomere length analysis to rule out dyskeratosis congenita or another telomere disorder.
If another form of physical dysmorphology is present, consider medical genetics consultation.
If the patient has anemia only, ring sideroblasts, and no dysplasia in lineages other than the erythroid, perform *ALAS2* genotyping if there is a family history of unexplained anemia or sideroblastic anemia or if the MCV is < 85 fL, and obtain a serum copper level as well as *SF3B1* mutation analysis.
If the patient has risk factors for copper deficiency (e.g., bariatric surgery, zinc supplement consumption), neutrophil vacuolization, or neurological changes (peripheral neuropathy, myelopathy), obtain a copper level.
If the patient has risk factors for human immunodeficiency virus (HIV) infection, perform HIV serology/polymerase chain reaction.
If the patient consumes more than one alcoholic drink per day, consider a 30-day trial of alcohol abstinence (but beware of alcohol withdrawal).
If the patient has isolated neutropenia (i.e., without anemia or thrombocytopenia), with a normal karyotype and without extensive marrow dysplasia, carefully review medications and supplements and consider obtaining a T cell receptor gene rearrangement assay; further rheumatologic evaluation may be useful.
If the patient has isolated thrombocytopenia, with a normal karyotype and without extensive megakaryocyte dysplasia, consider assessment for consumptive coagulopathy and empirical trial of immune thrombocytopenia therapy (e.g., corticosteroids).

MCV, mean cell volume; *MDS*, myelodysplastic syndromes

Sources: adapted and modified from Steensma DP [26] and Steensma and Stone [54]

somewhat arbitrary and have not been conclusively linked to outcomes [26, 27•]. If a MDS-associated karyotypic abnormality is present in a case with ambiguous morphological findings, then this gives increased confidence about an MDS diagnosis, but some karyotypic abnormalities such as trisomy 8, loss of the Y chromosome, or loss of the long arm of chromosome 20 are not specific enough to seal a diagnosis of MDS and are not recognized as disease-defining by the WHO. Surface antigen patterns are often abnormal in patients with MDS, and detection of these patterns by flow cytometry adds prognostic information, but clinical laboratories vary widely in their expertise in flow cytometry, so in many settings, flow cytometry is also not a reliable enough test to distinguish between MDS and other MDS mimics [28, 29].

Next-generation sequencing assessing for somatic mutations using panels of 20 or more MDS-associated genes is now routine in clinical practice when evaluating patients with unexplained cytopenias, at least in the USA, and the detection of mutation influences hematopathologists' interpretation of morphology [30, 31]. However, clinicians' comfort with interpreting results of such panels varies, as does the composition and validation of such panels, which can result in an additional degree of difficulty when evaluating patients with unexplained cytopenias and possible MDS. A task force of the American Society of Hematology is currently focusing on building greater consensus in somatic mutation testing both with respect to testing indications and panel composition.

Incidence and Prevalence of Unexplained Cytopenias

The global incidence and prevalence of unexplained cytopenias is not known with any confidence. Population series such as the Third National Health and Nutrition Examination Survey (NHANES III, which enrolled 39,695 Americans) indicate that anemia as defined by the WHO is very common in the aging population; anemia is present in 48–63% of those living in nursing homes, 11% of community-dwelling men age 65 and older and 10.2% of women in the same age cohort, and > 20% of those 85 and older (20.1% of elderly women and 26.1% of men) [32]. The incidence of anemia is even higher in non-Hispanic persons of African descent in the USA. Although NHANES III data indicated that nutritional deficiency accounts for about 1/3 of anemia in older persons, and renal insufficiency and inflammation for another 1/3 of these cases, at least 1/3 remains unexplained and > 15% of such patients have erythrocyte macrocytosis or other features suggestive of MDS [33]. Because most such anemia in the elderly is mild, patients may not undergo detailed evaluation including marrow biopsy or genetic testing, especially if they have serious comorbid conditions and a limited life expectancy.

The incidence of neutropenia in the general population is more difficult to estimate, as it is confounded by ethnic variations in the "normal" range of absolute blood neutrophil count [34]. In addition, so-called chronic idiopathic neutropenia, which is thought to be usually immune-mediated and is most commonly observed in middle-aged and older women, can be difficult to distinguish from other causes of neutropenia, including milder forms of congenital neutropenia [35]. However, data from NHANES III indicate that a neutrophil count $< 1 \times 10^9$/L is uncommon, seen in < 0.6% of adults [36].

Finally, thrombocytopenia is also very common in the general population, but while most unexplained thrombocytopenia is probably immunologically mediated, sensitive or specific tests for immune thrombocytopenia purpura are lacking [37, 38, 39••]. The lack of an inexpensive, widely available assay with good test characteristics that can reliably distinguish immune-mediated neutropenia or immune-mediated thrombocytopenia from other causes of these cytopenias is a major deficiency in contemporary hematology practice.

Outlook for ICUS and CCUS

It would be of considerable value to hematologists to be able to predict outcomes for patients with unexplained cytopenias and therefore to counsel patients appropriately about follow-up. In general, the rate of progression of thoroughly evaluated unexplained cytopenias to malignancy appears to be quite low, less than 1% per year. An important analysis incorporating molecular genetic testing into the evaluation of patients with unexplained cytopenias established that the risk of progression is much greater for clonal cytopenias of undetermined significance (CCUS) than ICUS [9••]. This series included a learning cohort of 683 patients investigated for unexplained cytopenia (435 of whom had a somatic mutation when studied with a 40-gene panel) as well as a validation cohort of 190 patients referred to a hematology center for suspected myeloid neoplasm. In these cohorts, only about 10% of those with ICUS had gone on to develop WHO-diagnosable myeloid neoplasms 5 years after initial evaluation, whereas more than 75% of those with CCUS had gone on to develop neoplasia by the same time point. The risk of progression to WHO-diagnosable disease was greatest in those who had multiple mutations rather than a single mutation, especially if one of the mutations was a splicing mutation, and progression was also more common in those in whom the size of the clone was substantial, especially VAF over 30%. Effectively, patients with CCUS with multiple mutations or larger clones have "MDS without dysplasia," since their natural history is similar to MDS despite the absence of definitive hematopathological changes. This series provides compelling evidence that results of next-generation sequencing can be used to help counsel cytopenic patients in clinical practice [39••].

Longitudinal Follow-up

There are no high-quality data to guide recommendations about the frequency of follow-up for patients with unexplained cytopenias [40]. Patients who have a history of solid tumors for which they have received chemotherapy or radiotherapy are at increased risk of therapy-related MDS or acute myeloid leukemia and require special monitoring, especially if mutations in *TP53* or *PPM1D* are present [41, 42].

In my own practice, I recommend monitoring blood counts every 6–12 months for those with ICUS and every 3–6 months for those with CCUS. I do not routinely perform bone marrows serially and reserve this for when the blood counts change, as the diagnostic yield of marrow biopsy is low in the absence of blood count changes. However, other clinicians do perform periodic marrow exams to assess blast proportion and dysplasia.

Implications of Monocytosis

Unexplained cytopenias associated with a relative or absolute increase in circulating blood monocytes carry a special risk of progression [43•]. Patients with monocytosis and with a flow cytometry pattern that reveals an excess of so-called classical monocytes are a particular risk of developing the myelodysplastic syndrome-myeloproliferative neoplasm

overlap disorder chronic myelomonocytic leukemia (CMML) [44]. Mutation testing can add further prognostic value in this setting. For example, in one series of 283 patients referred to a specialty center with unexplained monocytosis and evaluated with a DNA sequencing panel, the presence of a mutation was of greater prognostic value than conventional morphology, and mutations (especially in *ASXL1*, *CBL*, *DNMT3A*, *NRAS*, or *RUNX1*) were also associated with progressive cytopenias and increasing monocyte counts [43•].

Unanswered Questions

There is much yet to learn concerning patients with unexplained cytopenias, and many clinical and biological questions that are important to resolve remain. For instance, the relative contribution of other difficult-to-measure non-clonal causes of cytopenias such as autoimmunity in patients who have small mutant clones can be difficult to determine. Among patients with clonal cytopenias, triggers for subsequent clonal progression are unclear, as are the reasons why clonal populations can remain stable in the marrow for prolonged period [45•]. Acquisition of secondary mutations can be a potent driver of clonal progression in people with CHIP, but sometimes, new mutations are not detected at the time of progression [46, 47]. Interactions with the marrow microenvironment are likely to be important in reining in or promoting clonal expansion, as is the patient's own endogenous immunity [48, 49].

In the future, it may be possible to eliminate nascent clones before overt neoplasia develops, similar to how the multiple myeloma community has begun treating patients with high-risk smoldering myeloma (or even monoclonal gammopathy of undetermined significance) before overt myeloma develops [48]. This is a high-priority research task. However, the repertoire of drugs to eliminate myeloid clones is much more limited than those available for cytoreduction of plasma cell clones.

Patients with CHIP have a risk of cardiovascular events due to a pro-inflammatory interaction between the clonally derived monocyte-macrophage population and the vascular endothelium [50, 51••]. However, patients with CHIP are by definition not cytopenic, and it is currently unclear whether this CHIP-associated cardiovascular risk extends to CCUS, where the vascular risk may be modified by anemia, leukopenia, or thrombocytopenia. Attention to established cardiovascular risk factors (hypertension, dyslipidemia, smoking, diabetes mellitus) seems prudent for patients with CCUS as well as CHIP, and in the future, anti-inflammatory approaches may also help reduce vascular events in both populations [52, 53].

Conclusion

Unexplained cytopenias continue to confound hematologists and worry patients. Molecular genetic testing remains costly and is not universally available, but it can help distinguish between individuals with unexplained cytopenias who are at high risk of progression from those who are at lower risk. Additional tests are needed to help clarify the etiology of cytopenias in difficult cases, and development of treatments to eliminate emergent dangerous clones is critical.

Compliance with Ethical Standards

Conflict of Interest Dr. Steensma declares no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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