7. Dunleavy K, Pittaluga S, Shovlin M, et al. Low-intensity therapy in adults with Burkitt's lymphoma. *N Engl J Med*. 2013;369(20):1915-1925.

8. Panea RI, Love CL, Shingleton JR, et al. The whole-genome landscape of Burkitt lymphoma subtypes. *Blood*. 2019;134(19):1598-1607.

9. Alsharif R, Dunleavy K. Burkitt lymphoma and other high-grade B-cell lymphomas with or without MYC, BCL2, and/or BCL6 rearrangements. *Hematol Oncol Clin North Am*. 2019;33(4):587-596.

10. Bouska A, Bi C, Lone W, et al. Adult high-grade B-cell lymphoma with Burkitt lymphoma signature: genomic features and potential therapeutic targets. *Blood*. 2017;130(16):1819-1831.

DOI 10.1182/blood.2020005329

RED CELLS, IRON, AND ERYTHROPOIESIS

Comment on van Zeventer et al, page 1161

Does clonal hematopoiesis explain unexplained anemia?

David P. Steensma | Dana-Farber Cancer Institute

In this issue of *Blood*, van Zeventer and her colleagues in The Netherlands report mutational profiling in 676 anemic patients ≥60 years old and a group of age-matched nonanemic controls.[1] These study subjects were selected from the LifeLines population cohort, which includes 22 108 people in that age band, most in the northern provinces of The Netherlands. The prevalence of anemia in the LifeLines cohort, just >3%, is considerably lower than in the US National Health and Nutrition Examination Survey (NHANES) III cohort, which may relate to better population health in The Netherlands compared with the United States or germline genetic differences.

Many older people are anemic, and it is not always clear why. In the third US NHANES III cohort, for example, >10% of community-dwelling Americans >60 years of age were found to be anemic when using World Health Organization (WHO) hemoglobin cutoffs to define anemia, including >20% of the oldest old (ie, those over age 85).[2] About 2 out of every 3 NHANES anemia cases were due to nutritional deficiency, renal failure, or inflammation. The other one-third was considered "unexplained."

In the 15 years since the NHANES anemia prevalence data were reported, investigators have proposed various hypotheses to explain unexplained anemia in the elderly as well other "idiopathic cytopenias of undetermined significance" (ICUS).[3] These hypotheses include occult immune-mediated marrow suppression or premature blood cell destruction, stem cell "exhaustion," or undiagnosed myelodysplastic syndromes (MDS).

Definitive diagnosis of immune-mediated cytopenias remains difficult (eg, immune thrombocytopenic purpura remains a diagnosis of exclusion), whereas hematopoietic cell exhaustion is a multifaceted and rather ill-defined phenotype. However, there is certainly suggestive evidence that MDS is underdiagnosed. In 1 Israeli hospital, for example, geriatric patients who were admitted to a ward for patients with cognitive impairment and who were noted to have minor blood count abnormalities underwent in-depth evaluation; 15% ultimately were proved to have MDS.[4] Many elderly patients who might have MDS do not undergo full evaluation of mild cytopenias, especially very old patients with chronic health problems who are living in long-term care facilities in whom MDS, if diagnosed, would not be aggressively treated.

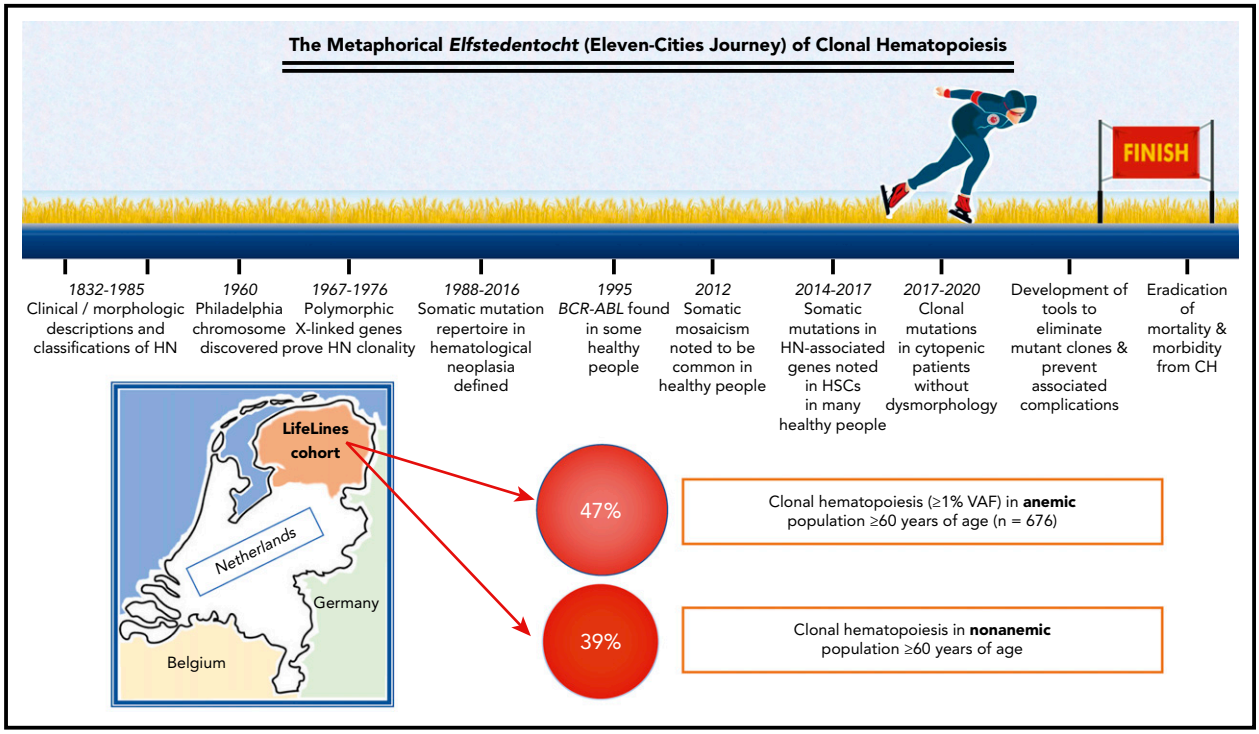
The high prevalence of anemia in the elderly is being reconsidered now that we know that somatic mutations are acquired in all tissues throughout the human lifespan, and that stable expanded blood cell populations derived from hematopoietic stem cells bearing acquired mutations that are associated with hematological neoplasia are present in almost everyone by middle age.[5,6] Most people with somatic mutations and clonal hematopoiesis have normal complete blood counts, however.[7] This remains true even when the mutations are in genes associated with MDS or other myeloid neoplasms and are present at a variant allele frequency (VAF) ≥2%, which is near the detection or reporting threshold of common clinical next-generation sequencing assays and was used to define clonal hematopoiesis of indeterminate potential (CHIP).[8]

In the Dutch series, somatic mutations in blood cells at ≥1% were more frequent in anemic individuals (46.6%) than in controls (39.1%), which is also higher than the prevalence of CHIP in previous series. The relatively small difference in prevalence between anemic people and controls, however, suggests that the mutations do not account for the majority of anemias in elderly people, which is underscored by the fact that there was no difference between groups in the prevalence of the 3 most common CHIP mutations: *DNMT3A*, *TET2*, and *ASXL1*. Instead, other genes were more commonly mutated in anemic persons, including *SF3B1*, strongly associated with ring sideroblasts and ineffective erythropoiesis, and the dreaded *TP53*.

With follow-up, most clonal populations were stable over time and exhibited little change in VAF, at least during the length of the monitoring period. It is an unresolved question why this stability occurs so commonly. Mutations such as DNMT3A R882H give hematopoietic cells a growth advantage compared with wild-type cells, yet clonal sweeping with complete dominance of hematopoiesis is uncommon in the absence of secondary mutations, for unclear reasons.

Interestingly, given the relationship between CHIP and inflammation,[9] mutations were more commonly detected in people thought to have anemia of inflammation compared with other anemia types, such as nutritional anemia. "Inflammation" is somewhat of a loosey-goosey concept, as there are many different inflammatory pathways and biomarkers. In the Dutch series, inflammation was defined by either elevated high-sensitivity C-reactive protein, unexplained leukocytosis, or an iron pattern consistent with inflammatory changes.

The Netherlands is the nation with the tallest people on the planet, and this new series underscores that size matters, at least when it comes to hematopoietic clones.



Milestones in understanding clonal hematopoiesis and preventing complications. The Elfstedentocht is an iconic 200-km ice-skating marathon along the canals, lakes, and rivers linking 11 cities in Friesland, the northernmost province in The Netherlands. Along with the neighboring province of Groningen, Friesland contributed most of the patients to the LifeLines population cohort and to the new study by van Zeveneter and colleagues, in which somatic mutations were found to be slightly more common in anemic elderly people compared to nonanemic controls, but common in both groups. The Elfstedentocht can only take place in the heart of winter after a long period of cold weather, when ice thickness reaches ≥15 cm along the along the entire skating route. Perhaps due to global warming, the event has not been held since 1997, and an "alternative" route has been substituted in colder Austria. Before 1960 and discovery of the Philadelphia chromosome, the genetic basis of hematological neoplasia was not understood. In the 1990s, molecular biology techniques permitted the observation that the same genetic variants associated with neoplasms, such as chronic myeloid leukemia, are sometimes present in blood cells in healthy people, albeit transiently, while the high prevalence and stability of certain types of expanded hematopoietic clones were definitively demonstrated in large-cohort and population-based studies of somatic mosaicism from 2012 onward. The next historical "milestones" along this lengthy journey of discovery and translation will include development of methods to eliminate dangerous mutant clones and prevent their complications, including cytopenias, inflammatory complications, and clonal progression. CH, clonal hematopoiesis; HN, hematological neoplasms; HSC, hematopoietic stem cells.

Larger clones with a VAF >5% or multiple mutations were associated with inferior overall survival, but smaller clones were not. There is a growing body of evidence that larger clone size is associated with increased risk of AML development, cardiovascular disease, and all-cause mortality.

The clinical concept of "clonal cytopenias of undetermined significance" (CCUS; ie, ICUS with a demonstrated mutation) is important, but CCUS includes a heterogeneous patient group. Let us imagine a 74-year-old woman who comes to a hematology clinic with a hemoglobin count of 10 g/dL, mean cell volume of 100 fL, unremarkable white count and differential, platelet count of 150 $\times$ 10^9/L, no obvious cause for her anemia such as B_{12} or folate deficiency, and a nondiagnostic marrow aspirate. On next-generation sequencing, she is found to have a *DNMT3A* mutation at 4% VAF. Is that clone by itself enough to explain her cytopenias? Probably not, nor do clonal mutations explain anemia for the majority of elderly patients.

However, a 75-year-old man with similar blood and marrow findings who has 3 or 4 mutations found on sequencing, including both a splicing mutation and *ASXL1* truncating variant with VAF >30%, effectively has "MDS without dysplasia." The detected clonal process likely does explain his anemia, and the subset of multiple-mutant high-VAF CCUS is also associated with a substantial risk of progression to WHO-defined myeloid neoplasia.[10]

In the future, it will be helpful to have tests that can distinguish in the individual patient whether clonal hematopoiesis and cytopenias are both present yet unrelated, or are causal and connected. Knowing which clones are at greatest risk of causing subsequent clinical complications based on VAF, specific allele pattern, and other parameters (ie, which patterns are the most dangerous) is also an important goal, which will then allow us to design mitigation strategies for these complications. We are a few skate lengths closer to achieving those goals, thanks to this new series (see figure).

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

REFERENCES

1. van Zeventer IA, de Graaf AO, Wouters HJCM, et al. Mutational spectrum and dynamics of clonal hematopoiesis in anemia of older individuals. *Blood*. 2020;135(14):1161-1170.
2. Guralnik JM, Eisenstaedt RS, Ferrucci L, Klein HG, Woodman RC. Prevalence of anemia in persons 65 years and older in the United States: evidence for a high rate of unexplained anemia. *Blood*. 2004;104(8):2263-2268.
3. Stauder R, Valent P, Theurl I. Anemia at older age: etiologies, clinical implications, and management. *Blood*. 2018;131(5):505-514.
4. Beloosesky Y, Cohen AM, Grosman B, Grinblat J. Prevalence and survival of myelodysplastic syndrome of the refractory anemia type in hospitalized cognitively different geriatric patients. *Gerontology*. 2000;46(6):323-327.
5. Steensma DP. New challenges in evaluating anemia in older persons in the era of molecular testing. *Hematology (Am Soc Hematol Educ Program)*. 2016;2016(1):67-73.
6. Young AL, Challen GA, Birmann BM, Druley TE. Clonal haematopoiesis harbouring

AML-associated mutations is ubiquitous in healthy adults. *Nat Commun*. 2016;7(1): 12484.

7. Jaiswal S, Fontanillas P, Flannick J, et al. Age-related clonal hematopoiesis associated with adverse outcomes. *N Engl J Med*. 2014; 371(26):2488-2498.

8. Steensma DP, Bejar R, Jaiswal S, et al. Clonal hematopoiesis of indeterminate potential and its distinction from myelodysplastic syndromes. *Blood*. 2015;126(1):9-16.

9. Libby P, Sidlow R, Lin AE, et al. Clonal hematopoiesis: crossroads of aging, cardiovascular disease, and cancer: JACC review topic of the week. *J Am Coll Cardiol*. 2019;74(4):567-577.

10. Malcovati L, Gallì A, Travaglino E, et al. Clinical significance of somatic mutation in unexplained blood cytopenia. *Blood*. 2017; 129(25):3371-3378.

DOI 10.1182/blood.2020005293

THROMBOSIS AND HEMOSTASIS

Comment on Marchetti et al, page 1171

Diagnosing HIT: the need for speed

Adam Cuker and Douglas B. Cines | University of Pennsylvania

In this issue of *Blood*, Marchetti et al describe a novel diagnostic algorithm for heparin-induced thrombocytopenia (HIT) based on the 4Ts score and 2 rapid immunoassays (IAs) that correctly classified >95% of patients within a 60-minute analytical window.[1]

HIT is a high-stakes diagnosis that must be made promptly and accurately. Failure to suspend heparin and initiate a nonheparin anticoagulant in patients with HIT is associated with an initial 6.1% daily rate of thrombosis, which may be limb- or life-threatening.[2] On the other hand, unnecessary treatment with a nonheparin anticoagulant in patients without HIT is costly and is associated with an incidence of major bleeding as high as 44%.[3]

The 2018 American Society of Hematology (ASH) HIT guidelines recommend a diagnostic algorithm for HIT based on the 4Ts score and the antiplatelet factor 4/heparin enzyme–linked immunosorbent assay (ELISA). HIT is excluded in patients with a low probability 4Ts score, whereas ELISA testing is advised in patients with an intermediate- or high-probability 4Ts score.[4] An important drawback of this algorithm is the time it takes. Because the ELISA has an analytic turnaround time (TAT) of 3 to 4 hours and is run in batch no more than once per day at most centers, same-day results are often not available. As a result, patients with intermediate- and high-probability 4Ts scores, many of whom do not have HIT, must be treated empirically with a nonheparin anticoagulant while awaiting test results.[5] In recognition of this limitation, the ASH guideline identified "integration of emerging rapid immunoassays into diagnostic algorithms" as a pressing research priority.[4]

Marchetti et al took this imperative to heart. Using Bayesian analysis like other HIT investigators before them,[5-7] they developed a diagnostic algorithm for HIT based on the 4Ts score, a rapid chemiluminescent IA (CLIA), and a rapid particle-gel IA (PaGIA) in 2 derivation cohorts from their center in Lausanne, Switzerland. They subsequently validated the algorithm in a separate, prospective cohort of consecutive patients with suspected HIT at the same institution. They used the heparin-induced platelet aggregation (HIPA) assay as the reference standard for HIT.[1]

The algorithm was highly effective in classifying HIT status. Of the 687 patients in the validation cohort, 655 (95.3%) were classified correctly by the algorithm. Only 12 patients (1.7%) were misclassified. All 12 of these patients tested negative by HIPA, but were classified as having HIT by the algorithm (false-positives). Importantly, there were no false-negatives. The remaining 20 patients (2.9%) were not classifiable by the algorithm and required additional testing to clarify HIT status.[1]

So how does the diagnostic algorithm of Marchetti et al stack up against the algorithm espoused in the ASH guidelines?[4] To address this question, we modeled the diagnostic accuracy of both algorithms in a hypothetical sample of 1000 patients with suspected HIT (see table). We assumed a prevalence of HIT of 7.9%, consistent with the prevalence observed in the validation cohort of Marchetti et al. As shown (see table), the Marchetti algorithm performed at least as well as the ASH algorithm. It correctly classified all 79 patients with HIT and 95.4% of patients without HIT, whereas the ASH algorithm correctly classified only 72 of the patients with HIT (91.1%) and 93.2% of patients without HIT. All told, the Marchetti algorithm misclassified 42 patients (4.2%), whereas the ASH algorithm misclassified 70 patients (7.0%).

Although superior diagnostic accuracy is an important plus of the Marchetti algorithm, it offers another key advantage

Test accuracy per 1000 patients with suspected HIT for 2 diagnostic algorithms

	ASH algorithm	Marchetti algorithm
Tests	4Ts score; IgG-specific ELISA (low threshold)	4Ts score; CLIA; PaGIA
True-positive	72	79
False-negative	7	0
False-positive	63	42
True-negative	858	879

Test accuracy is modeled on 1000 hypothetical patients with suspected HIT. We assumed a disease prevalence of 7.9%, the same prevalence as observed in the validation cohort of Marchetti et al. For the Marchetti algorithm, we assumed that the 2.9% of patients determined to be unclassifiable by the algorithm would be treated empirically for HIT; those patients ultimately found to have HIT by the reference standard were therefore classified as true-positives whereas those ultimately found not to have HIT were classified as false-positives. For the ASH algorithm, we used a sensitivity and specificity of 0.921 and 0.542, respectively, for the 4Ts score and 0.98 and 0.85, respectively, for the immunoglobulin G (IgG)–specific ELISA, the same values that were used in the ASH 2018 guideline on HIT.[4]