Anemia and adverse outcomes in the elderly: a detrimental inflammatory loop?

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Anemia is one of the easiest diagnoses in clinical practice, being based on a widely available and cheap laboratory parameter: hemoglobin concentration. In the near future, this diagnosis could become even simpler through a smartphone app.[1] Nonetheless, anemia is frequently overlooked, particularly when it is mild (e.g. hemoglobin values >10 g/dL but <12 g/dL or <13 g/dL in females and males, respectively) and no obvious symptoms are apparently associated. This typically occurs in the elderly, in whom other co-morbidities are often present, distracting the attention of physicians and caregivers.[2] Indeed, the prevalence of anemia in people aged >65 years is high, ranging from nearly 12% in those living in the community to more than 45% in institutionalized nursing-home residents.[2] While a mild anemia has been traditionally considered as a "physiological" consequence of aging, more recent studies have shown that the decline in hemoglobin is minimal, if any, in the "wellderly", namely people aging well without significant comorbidities.[3] Moreover, growing evidence suggests that anemia in the elderly is not an innocent bystander, being strongly and independently associated with a number of adverse outcomes, including cognitive decline, reduced physical performance, increased risk of falls and fractures, and even increased mortality.[2] In this issue of *Haematologica*, Wouters and colleagues report the results of the large Lifelines Cohort Study, confirming that anemia in the elderly is negatively associated with either quality of life or survival.[4] A key point of anemia in the elderly lies in the difficulty of establishing the etiology, which in turn should drive its management. While anemia in young people is generally due to a single cause (e.g. iron deficiency in premenopausal women with heavy menstrual bleeding), anemia in the elderly is often multifactorial, reflecting the typical multimorbidity of aged people.[5] This frequently makes it hard to dissect out the main mechanism leading to anemia in a given elderly individual. For example, iron deficiency in the elderly can be due to a mixture of malnutrition, malabsorption and bleeding, often aggravated by multiple medication use.[6] Such difficulty is even more pronounced in large epidemiological surveys, such as the Lifelines Study, in which only three general subcategories of anemia can be distinguished, based on a few available laboratory parameters: nutritional deficiencies, anemia of inflammation (also named anemia of chronic diseases), and "unexplained" anemia,[7] each of them accounting roughly for one third of cases. Notwithstanding these inherent limitations, the study by Wouters and colleagues points out anemia of inflammation as the most detrimental subcategory in the elderly, because of the strongest association with quality of life and mortality. This is somewhat at variance with the findings of other studies, in which subjects with anemia due to nutritional deficiency showed the worst prognosis.[8] Such a discrepancy reflects the uncertainty in the correct etiological classification of anemia in the elderly, as well as our limited knowledge in the field. The broad category of "unexplained anemia" well illustrates this gap, although it likely represents a heterogeneous group of conditions that cannot be adequately addressed by large epidemiological surveys because of the need for second level laboratory tests. Such conditions include androgen deficiency, vitamin D deficiency, unrecognized iron deficiency with apparently normal traditional biomarkers, impaired bone marrow response to erythropoietin, clonal hematopoiesis, and "inflammaging".[2] Of note, recent advances suggest that the last two conditions may share a relevant role in the pathophysiology of anemia in the elderly, both by inducing a low-grade chronic inflammatory status (Figure 1). Clonal hematopoiesis refers to age-related acquisition of somatic mutations in certain driver genes (e.g. *TET2*, *DNMT3A*, and *JAK2*) in hematopoietic stem cells, conferring them a competitive advantage and hence giving rise to a clonal progeny in the peripheral blood, at variance with normal polyclonal hematopoiesis.[9] Clonal hematopoiesis is detectable through next-generation sequencing studies in nearly 10% of 70-year old individuals without abnormalities of peripheral blood cell counts and, in these people, is a risk factor for subsequent development of myeloid neoplasms. This is generally associated with accumulation of multiple mutations, but the rate of progression appears as low as 0.5% per year. Such a condition has been termed clonal hematopoiesis of indeterminate potential (CHIP), since, in fact, most carriers of clonal hematopoiesis will never develop myeloid neoplasms. Nevertheless, individuals with CHIP have been found at increased risk of mortality due to cardiovascular events, rather than to hematologic complications.[10] Mounting evidence suggests that accelerated atherosclerosis in CHIP is associated with a systemic pro-inflammatory status driven by abnormal clonal leukocytes deriving from mutated hematopoietic stem cells.[11] On the other hand, "inflammaging" also contributes to a chronic upregulation of pro-inflammatory cytokines in the elderly. This phenomenon is thought to be the result of activation of the nuclear factor-κB/inflammasome pathway driven by the so-called damaged-associated molecular pattern, i.e., endogenous altered molecules and reactive oxygen species that accumulate with aging.[12,13] Reciprocal influences between CHIP and "inflammaging" are likely, for example considering that reactive oxygen species can also cause genomic instability, and that subclinical inflammation by itself can prime a detrimental vicious circle in tumorigenesis.[14] The exact proportions by which CHIP and "inflammaging" actually contribute to unexplained anemia in the elderly deserve further studies. Similarly, whether or not chronic subclinical inflammation, which is difficult to detect using classical biomarkers such as C-reactive protein, contributes to anemia through the same mechanisms as those associated with overt inflamma-

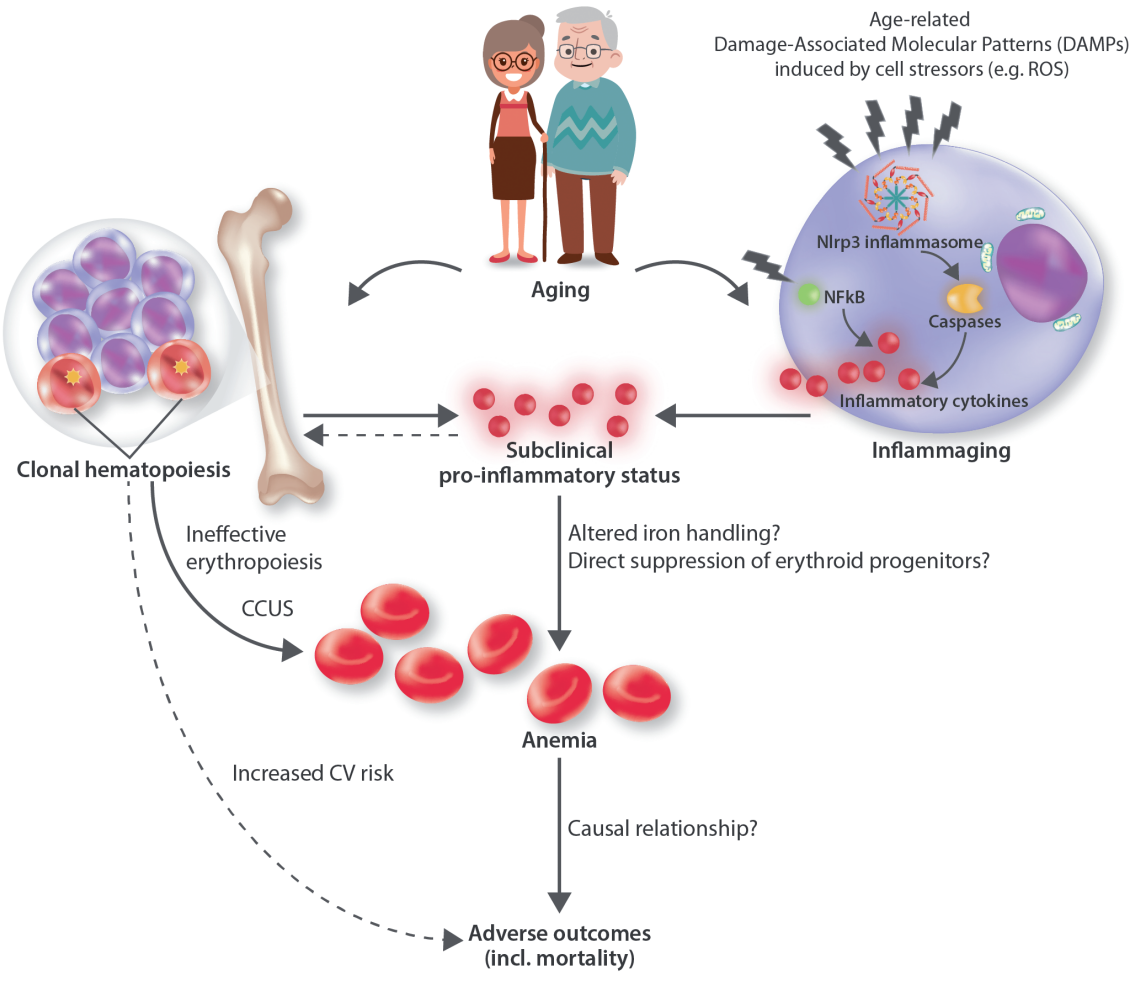

Figure 1. The complex relationship among aging, inflammation, and anemia. Clonal hematopoiesis and "inflammaging" are increasingly recognized as age-related conditions (see the text). Both are able to induce a mild chronic pro-inflammatory status, and can influence each other in a vicious circle. For example, chronic inflammation in the bone marrow can favor the development of somatic mutations in hematopoietic stem cells (dotted arrow). Clonal hematopoiesis and "inflammaging" are plausible explanations for at least a fraction of unexplained anemia in the elderly, likely through mechanisms similar to those involved in the pathophysiology of the classical anemia of *overt* inflammation. It is worth noting that clonal hematopoiesis is also able to induce anemia because of ineffective erythropoiesis. When cytopenia occurs in association with clonal hematopoiesis, it is termed clonal cytopenia of undetermined significance. Anemia in the elderly is strongly and independently associated with adverse outcomes, but whether or not this relationship is actually causal remains to be demonstrated. CV: cardiovascular; CCUS: clonal cytopenia of undetermined significance.

tion (e.g. hepcidin-driven iron-restricted erythropoiesis and cytokine-mediated suppression of erythropoiesis) remains to be demonstrated. Of note, clonal hematopoiesis can also cause anemia because of ineffective erythropoiesis, since progenitors carrying certain mutations can have a proliferative advantage over normal hematopoietic stem cells, but are less able to differentiate adequately.[15] Indeed, clonal hematopoiesis has been identified in a high proportion (64%) of elderly with unexplained cytopenia, for example with anemia not fulfilling all the morphological and cytogenetic criteria for the diagnosis of myelodysplastic syndrome.[16] In such cases, the term "clonal cytopenia of undetermined significance" has been proposed.[9]

Finally, a key point to be kept in mind when thinking about anemia in the elderly is that its robust association with mortality, even independently of the many possible confounders, does not prove a causal link, because of inherent limitations of observational epidemiology.[2] The only way to prove causality definitively would be the reduction of mortality and/or other adverse outcomes after successful correction or amelioration of anemia. The increasing number of promising anti-anemic drugs that are entering the clinical arena, including hepcidin antagonists,[17] novel iron formulations,[18] activin receptor IIA ligand trap,[19] and hypoxia inducible factor stabilizers,[20] may represent an unprecedented opportunity to clarify this crucial point in the near future.

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Targeting a major hub of cell fate decisions – the mitochondrial-associated membrane

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Great progress has been made in the treatment of human cancer but, unfortunately, there remains an abundance of treatment failures due to primary therapy resistance and/or the emergence of drug refractory clones. This is certainly true for acute leukemias, which are the most common malignancies in children and while they make up a smaller fraction of cancer in adults, their impact is substantial given the poorer outcome. Furthermore, the high doses of cytotoxic agents that are often used in therapy are associated with short- and long-term side effects. Thus, there is an urgent need to develop novel therapeutic approaches to improve outcome and decrease side effects. One such strategy, taken by Koczian and colleagues and described in this issue of *Haematologica*, is to augment the effectiveness of conventional agents.[1] They report that the use of the small molecule inhibitor of protein disulfide isomerase (PDI), PS89, has a significant impact on the effectiveness of cytostatic agents used routinely in the therapy of acute leukemias. The model that emerges is that PS89 amplifies the apoptotic stimulus induced by cytotoxic therapy, thereby allowing for increased efficacy at lower doses, through modulation of proteins at the mitochondrial-endoplasmic reticulum (ER) interface. The agent itself has poor pharmacokinetic properties limiting *in vivo* examination, but the results indicate substantial benefit and a wide therapeutic index. Much work remains to be done but the results emphasize the opportunity to target a unique intracellular sub-compartment that plays a key role in cell fate decisions: the interface between the ER and mitochondria.

Mitochondria are multifaceted organelles responsible for an array of cell functions critical for energy production, redox balance, adaptation to cell stress, and activation of the intrinsic apoptotic pathway. They make up 20% of the cytoplasmic volume of a cell and are dynamic, motile structures constantly altering shape through fission and fusion. These alterations involve two lipid bilayers that make up the inner membrane forming cristae (containing membrane-bound enzymes involved in oxidative phosphorylation) which enclose the matrix, and the smooth outer membrane. Mitochondria make important contact with other organelles, particularly the ER, which is in direct contacts with 20% of the mitochondrial surface. Changes in energy metabolism related to cancer, the so-called Warburg effect, have received renewed interest, especially with the discovery of "oncometabolites", but changes that occur at the mitochondrial-ER interface are also critical in controlling mitochondrial metabolism and cell fate decisions.[2]

The mitochondrial-ER interface, commonly referred to as the mitochondria-associated membrane (MAM), is a proteinaceous tether facilitating bidirectional communication between the two organelles controlling the balance between survival and death.[3,4] The exchange of metabolites and contact at the interface controls energy produc-