POINT-COUNTERPOINT

blood advances

COUNTERPOINT Stem cell donors should not be screened for clonal hematopoiesis

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Peripheral blood is continuously generated from hematopoietic stem cells (HSCs), which are defined by their dual capacities for sustained self-renewal and multilineage differentiation. Normally, a diverse pool of HSCs is maintained in homeostatic balance and contributes to polyclonal hematopoiesis. Expansion of HSC clones during normal aging is termed clonal hematopoiesis (CH) and was initially described based on skewed X chromosome inactivation.[1,2] Somatic clonal hematopoiesis marked by the presence of leukemia-associated point mutations using next-generation sequencing[3] and copy number alterations using single nucleotide polymorphism arrays[4,5] was subsequently described in the blood of aging individuals without hematologic abnormalities. Retrospective studies in population-level cohorts defined the scope of CH and identified specific links to adverse clinical outcomes, including an increased relative risk of hematologic malignancies,[6,7] increased risk of ischemic cardiovascular disease and stroke, and elevated all-cause mortality.[6,8] Laboratory models have directly linked CH to accelerated atherogenesis, mediated by potentiated immune/inflammatory activity in the mature immune cell progeny of CH clones.[8,9]

CH can be transferred from donor to recipient during allogeneic hematopoietic stem cell transplantation. A single-institution observational study of 552 consecutive hematopoietic stem cell transplantation recipients showed definitive proof of concept that expanded clones present in the blood of older donors can engraft in recipients and can be found in patients with persistent unexplained cytopenias.[10] A retrospective study of 500 donors age 55 or older found that *DNMT3A*-mutated donor CH was associated with increased chronic graft-versus-host disease (GVHD) and decreased risk of relapse among those not in remission at the time of transplantation.[11] However, neither of these studies systematically examined the impact of transplant-specific biological variables that could influence CH-related outcomes, including growth factor mobilization, prolonged systemic immunosuppression, chronic inflammation, or infections.

On the basis of scientific, technical, and ethical considerations, systematic screening of candidate donors for the presence of CH cannot yet be justified. Such a screening program would, at a minimum, require consensus guidelines that fulfill several basic criteria: (1) a uniform definition of clonal hematopoiesis, (2) a clear link between CH and transplant-related outcomes, and (3) a combined strategy for clinical actionability in donors and recipients. Importantly, the ethical implications and clinical obligations related to return of screening results to candidate donors create a high threshold of recipient benefit that must be met before implementation of systematic donor screening. With publication of adequately powered studies that define clear associations between CH and recipient outcomes, however, screening for CH may emerge in the future as a rational component of donor selection.

Defining CH

CH describes a clonal expansion of hematopoietic cells, without specific regard to its cause or to the presence of hematologic disease. Age-related CH has been used to refer to CH associated with any acquired clonal genetic alteration,[12] whereas CH of indeterminate potential (CHIP) has been used more specifically to define CH with mutations at variant allele fraction $\geq$2% involving a more restricted set of candidate driver genes that is identified in individuals with normal peripheral blood counts and no morphologic evidence of hematologic malignancy.[13] To engage the concepts in their most broad context, we will avoid provisional nomenclature and herein use “clonal hematopoiesis" or "CH” to refer to detectable clonal expansions in candidate stem cell donors.

Genetic heterogeneity

CH does not circumscribe an overarching, unified biological state. *DNMT3A* and *TET2* are the most commonly affected genes, composing two-thirds of CH, and the subset of patients with mutations in *DNMT3A* and *TET2* drive the set of reported clinical associations described previously. *ASXL1* is recurrently mutated in CH, albeit less commonly than *DNMT3A* and *TET2*, and has not been consistently associated with clinical outcomes. Other gene mutations, such as *JAK2*, *SRSF2*, *U2AF1*, and *SF3B1,* are even less common but may confer a higher risk of myeloid transformation, particularly in the setting of cytopenias.[14,15] Similarly, *TP53* and *PPM1D* mutations are most common in CH that arises after exposure to cytotoxic chemotherapy, and have only been shown to have clinical impact in that setting.[16,17] Still other genes that are reportedly mutated in CH, such as *MLL2*, *SETD2*, *CREBBP*, and *SETDB1*,[13] are rarely mutated in myeloid malignancies and may be enriched for spurious variants resulting from alignment artifacts, relaxed variant calling thresholds aimed at detecting small clones, or passenger mutations related to large gene size. These variants remain unvalidated, with limited to no functional or clinical data to support biological relevance in CH. Although permissive definitions of CH may be useful for research applications, screening would require a clinically grounded definition of CH that accounts for technical and biological heterogeneity to minimize the risk of false positives and inappropriate extrapolation.

Size of mutant clone

Clone size can be estimated based on the variant allele fraction (VAF) of a somatic mutation, where VAF is defined as the relative mutation abundance in the sample (number of variant reads/numbers of total reads). The lower limit of VAF detection is, in turn, dependent on several variables, including the type of sequencing platform, the complexity of the input library (the number of unique DNA molecules that are sequenced in a sample), and depth to which a sample is sequenced. The published evidence regarding the clinical implications of CH is largely based on a VAF cutoff of 2% to 5%, related to the challenge of distinguishing signal from noise (ie, mutation from artifact) at lower levels using whole exome and standard targeted exon sequencing platforms. However, clonal hematopoiesis reflects a continuum of mutation abundance, currently defined more by technical limitations than a biologically relevant threshold.

More recently, sequencing technologies that incorporate unique molecular identifiers, or molecular barcodes, have supported detection of clones with much lower abundance by enabling computational correction of low-level sequencing artifacts.[18,19] At their theoretically optimal level of performance, platforms that use unique molecular identifiers could remove all sequencing artifacts and detect variants at extremely low levels. In fact, recent studies have reported levels of detection to VAFs of 0.03% and proposed that clonal hematopoiesis is a near-ubiquitous phenomenon even in young individuals.[20] However, even sequencing methods that are designed to correct errors are not completely free of artifacts. As such, variants reported at very low levels should still be viewed with caution, especially when involving residues, domains, or genes not commonly mutated in leukemia. Further, the ability to identify variants using ultra-high-sensitivity sequencing approaches clearly outpaces clinical research, and the biological implications of small clones remain largely undefined. Incorporation of screening for CH into donor evaluation would thus require adoption of a universal definition of CH that encompasses the spectrum of recurrently mutated genes, mutation distribution within genes, and clone size, as well as uniform technical standards for next-generation sequencing platforms and interpretation of sequencing results.

Association with transplant clinical outcomes

Donor CH has not been linked to inferior overall outcomes in transplant recipients. Based on studies in nontransplant populations, it might be assumed that donor-engrafted CH would predict worse recipient outcomes after transplantation because of increased risk of both malignant complications, such as donor cell leukemia (DCL), and nonmalignant complications, such as cardiovascular disease. Although case reports have identified DCL that can be traced back to donor CH, DCL is a rare and late complication of transplantation that overall does not have a significant effect on posttransplant survival.[21-23] Whether the risk of leukemic transformation of donor-engrafted CH exceeds that of CH in native hematopoiesis (reported as 1% per year[6]) has not been evaluated.

The most common adverse outcomes after transplantation are relapse, GVHD, and infection, with cardiovascular complications such as ischemia and thrombosis being less frequent.[24] To date, there is only one published study comparing outcomes in recipients of clonal grafts compared with recipients of non-clonal grafts.[11] This study found an increased cumulative incidence of chronic GVHD, but no impact on infection, unexplained cytopenias, or overall survival. Two recently presented abstracts from studies of similar size reported contradictory findings, with one study showing an increased risk of acute GVHD but not chronic GVHD,[25] and a second finding no impact on post-transplant outcomes whatsoever.[26] With three studies showing divergent results, there is a clear lack of evidentiary consensus about the clinical implications of donor CH in transplantation. As such, initiation of donor screening would currently rely solely on extrapolation from retrospective non-transplant studies rather than on a reproducible demonstration of specific adverse transplant-related outcomes.

Could donor-engrafted clones have immunologic consequences that are favorable for recipient outcomes? In addition to an adverse effect on GVHD risk, Frick et al observed that CH in donors may be associated with a lower risk of relapse in the subset of patients not in remission at the time of transplant.[11] Together, these findings suggest that donor-engrafted CH may potentiate global immune/inflammatory activation in allografts, resulting in concomitant augmentation of graft-versus-leukemia and graft-versus-host activity. Mechanistically, this effect could be mediated by pleiotropic effects of CH mutations in mature myeloid or lymphoid lineages because CH can display multilineage tropism in native hematopoiesis.[27,28] Consistent with this possibility, a case report has described the augmented antileukemic activity of insertional inactivation of *TET2* in the setting of chimeric antigen receptor T-cell therapy.[29] The possibility that donor CH could confer beneficial transplantation outcomes in a subset of recipients with high-risk disease further argues against premature initiation of screening based on limited retrospective analyses or extrapolation from nontransplant studies.

Implications for donors

Screening for CH among potential stem cell donors raises several ethical and operational implications related to disclosure of results. At a population level, CH is associated with risks of cardiovascular disease, stroke, and hematologic malignancy, but there are no evidence-based strategies available to mitigate this risk. There may be psychological and emotional effects to a "diagnosis" of CH in healthy donors, even when the finding is disclosed by a practitioner who is experienced in conveying the nuances of the condition.[30] There are currently no accepted consensus guidelines for how to follow CH in healthy individuals, no systematic knowledge about the negative effects of disclosure of unactionable results of CH testing, and no published infrastructure at National Marrow Donor Program or Deutsche Knochenmarkspenderdatei for implementing the return of CH screening result to candidate donors.

Some genes that are somatically mutated in CH, such as *TP53*, can also be mutated in germline cancer predisposition syndromes,[31,32] and incidental identification of pathogenic germline variants during donor CH screening could occur. Finding such a mutation in an otherwise healthy donor would trigger the need for confirmatory testing using definitive constitutional reference tissue and referral for genetic counseling. With ongoing efforts to maximize donor recruitment by minimizing barriers and consequences to registry signup, a clearly articulable evidence-based benefit to recipients should first be defined.

Summary

The current level of evidence is insufficient to support implementation of prospective screening of donors for clonal hematopoiesis. Further, without clear consensus guidelines for interpretation of results, standardized methodological approaches, and clinical infrastructure for donor notification and counseling, there is a potential for unintended negative consequences of screening. Because our conclusion is based on the lack of demonstrated actionability of a positive screening result, we further emphasize that the pretest likelihood of finding CH in a potential donor, informed by older age or relatedness, does not factor into our recommendation. Additional retrospective and prospective studies of donor CH that are adequately powered to engage the scientific questions outlined here may clarify the magnitude of risk or benefit associated with donor CH, thereby prompting reevaluation of this assessment.

Authorship

Contribution: C.J.G. and R.C.L. wrote the manuscript.

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