Genetic panels in young patients with bone marrow failure: are they clinically relevant?

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Bone marrow failure can be acquired or inherited. Acquired forms are usually immune-mediated; inherited forms may be due to DNA repair defects (Fanconi anemia), ribosomopathies (Shwachman-Diamond and Diamond-Blackfan anemia), telomere defects (dyskeratosis congenita), and a smattering of other germline mutations that lead to cytopenias and predispose to myelodysplastic syndromes (MDS). Acquired severe aplastic anemia (SAA) is treated with allogeneic hematopoietic stem cell transplantation (HSCT) or immunosuppressive therapy (IST) in patients without a suitable donor. Inherited bone marrow failure syndromes are treated with HSCT if they have severe marrow failure, as IST is ineffective. Importantly, the HSCT conditioning regimen is not the same for all of these disorders. Patients with Fanconi anemia must receive a less intensive HSCT conditioning regimen. Inherited forms of bone marrow failure almost always present in the first decade of life and often, but not always, have either physical stigmata or a family history that is suggestive of the diagnosis. However, in many cases the overlap in clinical presentation and bone marrow features of inherited and acquired bone marrow failure syndromes and pediatric MDS can present a diagnostic challenge. A reliable method to distinguish between inherited and acquired bone marrow failure syndromes would accelerate the diagnosis and could influence the choice of therapy and/or the choice of donor for HSCT.

In this issue of *Haematologica*, Keel *et al.*[1] applied a multiplex targeted capture assay to investigate 63 genes in 208 children and young adults with AA (aplastic anemia) or MDS. These genes had all previously been described in patients with inherited bone marrow failure syndromes. All patients underwent HSCT between 1990-2012. The reported mutation frequencies in each cohort were commensurate with previous reports with similar sequencing methodology.[2-6] A thoughtful and extensive characterization of the cohort retrospectively reviewed the patient's chart history and physical exam characteristics as well as clinical outcomes.[1]

The AA cohort had 53 pediatric (age ≤18 years) and 45 young adult (age >18 years but ≤40) patients. All of them met the criteria for severe disease at the time of HSCT. Physical anomalies were present in 11% of the AA patients and 40% had a family history suggestive of inherited conditions. The authors found known mutations in 5 of 98 AA patients. The mutations identified for the 5 patients in the AA group included *DKC1* (n=2), *MPL* (n=2), and *TP53* (n=1). These all represent known constitutional mutations which have previously been described to result in the clinical syndromes of dyskeratosis congenita,[7] congenital amegakaryocytic thrombocytopenia,[8] and Li Fraumeni,[9] respectively.

The MDS cohort had 46 pediatric (age ≤18 years) and 64 young adult (age >18 years but ≤46) patients. Physical anomalies were present in 24% of patients, with family histories relevant in 52% of the patients. The authors found mutations in 15 of 110 MDS patients. The mutations identified were constitutional in 14 out of these 15 patients. They included compound heterozygous mutations in *FANCA*, *MPL*, *RTEL1*, and *SBDS* with heterozygous mutations identified in *GATA2*, *RUNX1*, *TERT*, *TINF2*, and *TP53*. These have been reported to cause Fanconi anemia,[10] congenital amegakaryocytic thrombocytopenia,[8] dyskeratosis congenita,[7] and Shwachman-Diamond syndrome.[11] Additionally mutations in *GATA2* and *RUNX1* are known to have an inherited predisposition to leukemia and MDS.[12,13] The fifteenth patient with mutated bone marrow DNA carried a heterozygous mutation in *RUNX1*. The authors note a comparison with bowel and skin DNA (wild-type *RUNX1*) in the patient that ultimately identified this as a somatic mutation.[14]

The strengths of this work by Keel and colleagues relate to the large number of samples in their biorepository and the meticulous work in performing the genetic analysis. In terms of clonal hematopoiesis, the authors used a panel which focused on genes that are previously described in inherited as well as acquired AA and MDS. Newer techniques and sequencing data could possibly have identified even more genes associated with these diseases of marrow failure. The relevance of a panel such as this to the marrow failure population at large is that it can identify markers (even somatic) of clonal hematopoiesis. In AA, in particular, this clonal hematopoiesis has been closely linked to the evolution of late clonal disorders, including MDS, leukemia, and PNH, even after successful treatment with IST. The detection and close monitoring of somatic mutations may help with predictions of outcomes and earlier diagnosis of clonal evolution, all leading to better management of patients with AA.

The authors of the study under discussion attempted to retrospectively ascertain a "pre-test" probability of inherited syndromes through a review of the chart history and physical anomalies, but the amount of incomplete clinical data may limit the wider applicability. Also, since the biorepository used patients who received an allogenic HSCT, it likely excluded patients with moderate aplastic anemia. The authors concluded that the history and physical examination did not identify a subset of patients with underlying mutations. However, in this small cohort, conclusions were not made based on age; four of the 5 AA patients with germline mutations were less than 10 years of age, and the 33-year-old patient had a known family history consistent with dyskeratosis congenita. Thus, given the added cost of these gene panels and the low yield in older patients with no family history or physical stigmata, it may be more cost effective to restrict their use to patients under 18 years of age. Further stratification may be possible after screening for a PNH clone. It has been previously described that the presence of a PNH

clone essentially rules out inherited conditions, as this is a marker of acquired disease.[15] Accordingly, in patients found to have a PNH clone by flow cytometry, none had a germline mutation. Thus, it remains unclear as to whether gene panels will be useful, especially in patients beyond their second decade of life, and particularly if they have a PNH clone.

Another potential benefit of targeted gene panels may relate to HSCT donor selection. As the use of alternative donor sources (matched unrelated donors, haploidentical donors, and cord blood) increases, we need assurances that we are not transplanting defective stem cells. One could argue to always use unrelated donors. However, there is ample evidence that time to treatment matters in severe pancytopenia. Thus, the use of a related donor without increased susceptibility to marrow failure would decrease the time to HSCT without having to search for an unrelated donor.

In conclusion, the study by Keel and colleagues is an important first step in helping to define the incidence and clinical importance of germline mutations in young patients with severe bone marrow failure. Future prospective studies and improved technology are needed before a more widespread application of targeted gene panels and/or genome sequencing can be recommended in routine clinical practice.

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Autotransplants in older multiple myeloma patients: hype or hope in the era of novel agents?

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Multiple myeloma (MM) is a malignant disease characterized by the proliferation of clonal plasma cells (PCs) in the bone marrow (BM), and typically accompanied by the secretion of monoclonal immunoglobulins that are detectable in the serum and/or urine. Increased understanding of the genetic alterations, the interactions between malignant PCs and the BM niche and their role in disease progression and the acquisition of therapy resistance, has helped in the development of novel agents, used in combination with cytostatic therapy, including autologous stem cell transplantation (ASCT). The most common indication for ASCT in Europe and the United States is MM, nevertheless elderly patients are often excluded from ASCTs, due to the patients' and/or physicians' choices, subjectivity towards its effectiveness in older cohorts, large prospective studies mostly lacking in elderly cohorts, the effectiveness and broad availability of novel agents and the fear of transplant-related toxicity.[1,2]

The median age of MM patients at diagnosis is approximately 70 years, with 60% aged 65 or older and ~30% being older than 75 years. The transplant age cutoff has been proposed to be <70 years. In clinical trials for ASCT, the age cutoff is even lower, and commonly 65 years, even if the feasibility of ASCT is established as being up to the