EDITORIAL

Unveiling amphiregulin: a blood-based biomarker for graft-*versus*-host disease risk assessment and monitoring

Sung Won Choi

University of Michigan, Michigan Medicine, Blood and Marrow Transplantation Program, Ann Arbor, MI, USA

Correspondence: S.W. Choi
sungchoi@umich.edu

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Over recent decades, our understanding of the genesis and pathophysiology of acute graft-*versus*-host disease (GvHD) has advanced significantly. However, a key challenge has remained in the field: for clinicians to predict GvHD-related mortality accurately based on symptom severity alone. The severity of symptoms often does not reflect the mortality risk associated with acute GvHD adequately, particularly because of the intricate dynamics of the body's response to therapy and the dual nature of the beneficial graft-*versus*-leukemia effect. In the quest to address these key clinical dilemmas, a new era of leveraging blood-based biomarkers emerged as a promising avenue for non-invasive risk assessment and monitoring of acute GvHD.[1]

In the early 1990s, the focus rested primarily on pro-inflammatory cytokine markers as potential indicators of GvHD (e.g., TNF, IL-2R). Entering into the 2000s, sophisticated '-omics' techniques, such as comprehensive profiling of plasma proteomes, substantially accelerated the ability to identify markers with heightened sensitivity and specificity. The first validated blood-based biomarkers for acute GvHD were combined into a four-marker panel (IL-2Rα, TNFR1, IL-8, and HGF).[2] Since then, biomarkers took center stage, including Reg3α,[3] ST2,[4] and amphiregulin (AREG).[5] This enhanced marker identification has not only enriched the grading criteria for acute GvHD but has also paved the way for risk stratification strategies. Notably, standardized grading criteria and risk stratification methods, such as the Minnesota GvHD Risk Score[6] and Ann Arbor Biomarker Score,[7] have become instrumental in assessing GvHD severity and prognosis. These advancements underscore the dynamic evolution of our diagnostic capabilities, further deepening insights into the underlying mechanisms of GvHD.

Nonetheless, predicting disease onset and subsequent disease course, including response to treatment, remains a grand challenge in medicine, limiting the full potential of personalized medicine. Given the complex dynamic systems involved, detection of disease at its earliest, pre-symptom stage is often complicated by changes occurring over time based on new, ongoing data about the disease process. The once "snapshot" paradigm of measurement in the transplant field has evolved through analysis of frequent, non-invasive blood samples obtained longitudinally at designed timepoints within a framework of robust biorepositories or multicenter clinical trials with well-annotated clinical data. Analyzing samples derived from the Chronic GvHD Consortium and Mount Sinai Acute GvHD International Consortium,[8] followed by Blood and Marrow Transplant Clinical Trials Network 0302 and 0802 studies, Holtan and colleagues validated initial AREG biomarker investigations by confirming the prognostic significance of this protein in acute GvHD.[9] They have now comprehensively evaluated the utility of AREG as a monitoring biomarker in two recent clinical trials.[10] The first trial investigated urinary-derived human chorionic gonadotropin/epidermal growth factor (uhCG/EGF) in supportive care for high-risk acute GvHD patients enrolled in a single-center setting (NCT02525029). The second trial, known as the REACH1 study, involved patients with steroid-refractory acute GvHD enrolled in a multicenter setting (NCT02953678).

A key observation from the study by Holtan *et al.*, published in this issue of *Haematologica*,[10] was the consistency of the performance of AREG across different measurement platforms. The correlation of AREG levels between enzyme-linked immunosorbent assay and microfluidic immunoassay platforms demonstrated a high degree of agreement, highlighting the potential feasibility of the implementation in clinical laboratories. The analyses yielded several notable findings. In patients achieving a complete response at day 28 of uhCG/EGF therapy, AREG levels exhibited a significant decrease from baseline to day 56 (mean, 98 *vs*. 32 pg/mL, P=0.006). Conversely, AREG levels remained relatively stable in patients with partial or no response to hCG/EGF treatment. The identification of a specific AREG cutoff (≥212 pg/mL) at study baseline provided a valuable tool for

risk assessment. Patients with AREG levels exceeding this threshold faced a markedly higher risk of rapid mortality within a median of 62 days.

Interestingly, similar trends in the data were observed in the REACH1 study. Patients who achieved a complete response experienced a substantial decrease in AREG levels from baseline to day 56 (mean, 174.7 *vs*. 63.6 pg/mL, *P*=0.007). This trend also extended to patients treated with ruxolitinib who showed a very good partial response or partial response. In contrast, patients with progressive disease did not have any significant changes in AREG levels over time. Multivariate analyses further highlighted the importance of response at day 28 and baseline AREG as independent predictors of survival in both cohorts. In the uhCG/EGF study, patients with high baseline AREG faced a 4.2-fold increased risk of mortality, while those treated with ruxolitinib and had high baseline AREG had a 2.7-fold elevated risk of death.

Using these two study cohorts, Holtan and colleagues established a universal AREG cutoff of ≥330 pg/mL, unveiling AREG as a potential early mortality risk assessment tool. This finding has particular relevance in clinical scenarios in which interpreting response may be challenging due to confounding variables, such as medication side effects, gastrointestinal infections, or other dietary alterations. The investigation by Holtan and colleagues further delved into the complex dynamics of AREG, shedding light on its diverse physiological roles. First described in 1988 as a signaling molecule, AREG belongs to the EGF protein family and is integral to cellular processes, such as growth, differentiation, and survival. Produced by epithelial cells, fibroblasts, as well as immune cells, AREG binds to the EGF receptor on target cells, and has been shown to be a key player in type 2-mediated resistance and tolerance, including in murine GvHD biology.[11] Although elevated AREG levels are noted during acute GvHD, tissue expression patterns have varied. Recent evidence hinted at the involvement of immune cells in circulating AREG production during acute GvHD. Alloreactive CD4 T cells, for example, were found to upregulate AREG expression during murine GvHD. These findings, coupled with the observed correlation between circulating AREG and cell-bound AREG on various immune cell subsets suggest a complex interplay between immune cells and AREG.[12]

In conclusion, the study by Holtan *et al.* unveils AREG's role as a biomarker that closely aligns with risk stratification and clinical response monitoring in life-threatening acute GvHD. Being able to measure AREG levels reliaby across different measurement platforms holds promise for rapid adoption across institutions in which hematopoietic cell transplants are being performed. The integration of correlative biomarkers into the framework of clinical trial design represents a significant advancement in the field. Future research endeavors should validate these findings in real-time as well as examine AREG in different settings, such as haploidentical transplants, which may further improve our understanding of this biomarker's performance.

Disclosures

No conflicts of interest to disclose.

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