Application of Proteomics in Acute Graft-Versus-Host Disease Management: An Integrative Review and Nursing Implications

Nilesh Kalariya, RN, PhD, and Kelly Brassil, RN, PhD

Background: After allogeneic hematopoietic stem cell transplantation, one of the major barriers to clinical management of acute graft-versus-host disease (aGVHD) is a lack of reliable and validated noninvasive tests for diagnosis and prognosis. Proteomic studies have indicated a strong correlation between the level of certain body fluid proteins and clinical outcomes after aGVHD. Specific proteins have been identified that could be robust biomarkers for overall prognosis or for differential diagnosis of target organs in aGVHD.

Objectives: The authors aimed to evaluate the literature related to proteomic biomarkers that are indicated in the occurrence, severity, and management of aGVHD.

Methods: PubMed and CINAHL® databases were searched for articles published from January 2004 to June 2014. Eight articles matching the inclusion criteria were identified, and the findings of these articles were summarized and their clinical implications noted.

Findings: Proteomics appears to be a promising tool to assist oncology nurses and nurse practitioners with patient education, develop personalized plans of care to reduce morbidity, initiate communication regarding end-of-life decisions, and improve overall nursing management of the population of patients with aGVHD.

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The efficacy of allogeneic hematopoietic stem cell transplantation (HSCT), a treatment option for some hematologic malignancies, is often limited by graft-versus-host disease (GVHD). GVHD results from an immunologic attack on target recipient organs or tissues (e.g., skin, gut, liver) by donor allogeneic T-cells and leads to death in greater than 15% of these recipients of HSCT (Pasquini, Wang, Horowitz, & Gale, 2010). Acute GVHD (aGVHD) occurs in about half of recipients of allogeneic HSCT, can be very debilitating, and often indicates a poor prognosis (Ferrara, Levine, Reddy, & Holler, 2009; Jagasia et al., 2012). Steroids are the first-line treatment for aGVHD, but, among patients with steroid-refractory aGVHD, the long-term mortality rate can still be greater than 90% (Blazar, Murphy, & Abedi, 2012).

Advances in proteomics, the study of the structural and functional roles of various proteins, have identified a number of body fluid proteins that may serve as biomarkers for the management of various diseases (Good et al., 2007). The application of these biomarkers could transform current aGVHD management modalities (Paczesny, 2013), including predicting the development of disease, facilitating early diagnosis, improving assessment of treatment response, and predicting patient survival. For oncology nurses caring for patients with aGVHD, familiarity with proteomic testing and interpretation of the results of these tests to prognosis and treatment options is imperative when developing the plan of care for and with these patients.

The major barrier to successful clinical management of aGVHD is the lack of diagnostic and prognostic tests. Laboratory tests could help identify patients who may not respond to therapy and patients at high risk for morbidity and mortality, and it could allow for earlier opportunities to individualize treatment plans in complex cases. Validated physiologic indicators...
of patient survival rates could be used to facilitate end-of-life decisions. The discovery of reliable, noninvasive, and validated biomarkers could contribute to improved aGVHD management and decrease patient morbidity and mortality.

This review identifies proteomics biomarkers for aGVHD management, discusses the clinical relevance of proteomics for oncology nurses in managing aGVHD, and proposes future directions for evidence-based nursing practice.

Methods

An integrative literature review was completed to identify what is known about the use of proteomics for aGVHD identification, management, and prognostication. PubMed and CINAHL® databases were searched using key words aGVHD, proteomics, protein, biomarker, skin GVHD, gastrointestinal GVHD, serum, plasma, urine, saliva, and allogeneic hematopoietic stem cell transplant. Studies with human participants published from January 2004 through June 2014 were retrieved and screened to identify studies that (a) investigated proteins as aGVHD biomarkers, (b) determined the level of proteins in body fluids, (c) used body fluids collected noninvasively, and (d) validated preliminary findings with an independent set of patients. Eight studies from two research groups met inclusion criteria, and their findings were summarized and results analyzed for their implications for clinical practice (see Tables 1 and 2).

Literature Review

Many proteins associated with pathological changes, such as tumor necrosis factor (TNF) alpha and hepatocyte growth factor (HGF), are low-abundant circulating proteins that require highly efficient methods for detection. The studies included in this review employed one or more of the following methods: enzyme-linked immunosorbent assay (ELISA), intact protein analysis system (IPAS), and capillary electrophoresis–mass spectrometry (CE-MS). In ELISA, an enzyme is added to a test sample, such as blood or urine, to facilitate binding of the enzyme with the protein of interest. Successful binding creates a colored solution. The amount of color in the sample corresponds directly with the amount of the protein in the sample (Lequin, 2005). IPAS is a three-dimensional system that separates mixtures of proteins according to charge, hydrophobicity (water-repellent property), and molecular mass. Mass spectrometric analysis can then be carried out to study specific proteins of interest (Wang et al., 2005). In CE-MS, proteins or peptides are separated from each other on the basis of their charge-to-size ratio under the influence of an electrical field. Combining CE and MS enhances the selectivity and sensitivity of detecting proteins (Ramautar et al., 2012).

### TABLE 1. Candidate Proteins With Validated Diagnostic and Prognostic Significance for Management of Acute Graft-Versus-Host Disease

<table>
<thead>
<tr>
<th>Protein</th>
<th>Source of Body Fluid</th>
<th>Prognostic Significance</th>
<th>Diagnostic Significance</th>
<th>Assessment Method</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elafin</td>
<td>Blood</td>
<td>X</td>
<td>X</td>
<td>ELISA</td>
<td>Levine et al., 2012</td>
</tr>
<tr>
<td>IL-8</td>
<td>Blood</td>
<td>X</td>
<td>X</td>
<td>ELISA</td>
<td>Paczesny et al., 2009</td>
</tr>
<tr>
<td>KRT18</td>
<td>Blood</td>
<td>X</td>
<td>X</td>
<td>ELISA</td>
<td>Harris et al., 2012</td>
</tr>
<tr>
<td>REG3α</td>
<td>Blood</td>
<td>X</td>
<td>X</td>
<td>IPAS + ELISA</td>
<td>Ferrara et al., 2011</td>
</tr>
<tr>
<td>REG3α</td>
<td>Blood</td>
<td>X</td>
<td>X</td>
<td>ELISA</td>
<td>Levine et al., 2012</td>
</tr>
<tr>
<td>ST2</td>
<td>Blood</td>
<td>X</td>
<td>X</td>
<td>IPAS + ELISA</td>
<td>Vander Lugt et al., 2013</td>
</tr>
</tbody>
</table>

### Salient Features of Advances in Proteomics

Four salient features were identified in the articles included in this review. Body fluid appears to be an ideal noninvasive
tool for and source of biomarkers: elafin, a protein, is a potential candidate for managing skin GVHD; regenerating islet-derived 3-alpha (REG3α), HGF, and keratin 18 (KRT18) protein levels could be exploited to manage gastrointestinal GVHD; and suppression of tumorigenicity (ST2) is a protein that strongly predicts the survival of patients with aGVHD.

Body fluid is a noninvasive source of biomarkers. Paczesny et al. (2009) developed the first blood-based proteomic panel. Eight potential protein biomarkers for aGVHD were identified with the antibody microarray method from a complex mixture of thousands of circulating plasma proteins. The levels of these eight proteins were measured by ELISA in plasma samples from another set of recipients of HSCT. Following logistic regression analysis, the authors identified an optimal composite biomarker panel containing four proteins (interleukin-2 receptor alpha [IL-2Rα], TNF receptor 1 [TNFR1], interleukin-8 [IL-8], and HGF). This set of four proteins was able to discriminate between recipients of HSCT who developed GVHD and those who did not at the onset of clinical symptoms, and it provided prognostic information independent of aGVHD severity.

Urinary proteins have also been identified as an ideal source of noninvasive biomarkers. Two pioneering studies (Weissinger et al., 2007, 2014) identified a set of 31 urinary protein fragments (also referred to as polypeptides) that can predict the development of aGVHD. Using CE-MS, Weissinger et al. (2007) demonstrated that this set of polypeptides significantly distinguished aGVHD of grade 2 or higher prior to clinical diagnosis (sensitivity = 85%, 95% confidence interval [CI] [73.1, 87.9]; specificity = 76%, 95% CI [71.6, 79.4]). However, the identity of the 31 polypeptides was not reported in the study. In follow-up work, Weissinger et al. (2014) conducted a multicenter validation of an aGVHD-specific urinary proteomic classifier called aGVHD-MS17, which contains 17 polypeptides. Using logistic regression, the classifier predicted the onset of severe aGVHD 14 days before any clinical signs were observed (sensitivity = 82%, specificity = 77%). Multivariate regression analysis demonstrated that GVHD-MS17 positivity significantly predicted grade 3 or 4 aGVHD (p < 0.0001). These peptides are fragments derived from proteins, such as albumin, beta-2 microglobulin, cluster of differentiation 99, fibronectin, and various collagen alpha chains. They indicated the early presence of inflammation, activation of T cells, and changes in the extracellular matrix. They can also be used to detect initial signs of GVHD-induced organ damage.

These studies have provided the best validated demonstrations of accurately predicting aGVHD using body fluids as a noninvasive tool for identifying protein biomarkers. The identification of proteins or protein fragments has also demonstrated how proteomic studies could be essential to identifying specific physiologic changes that predict the potential for organ damage specific to aGVHD.

Biomarkers for detecting skin acute graft-versus-host disease: Paczesny et al. (2010) identified a plasma biomarker specific to skin aGVHD. The lead candidate protein in this study, elafin, was identified via ELISA and IPAS through validation with an independent cohort of patients. Elafin level in plasma samples was significantly higher in patients with the onset of skin aGVHD than in patients with skin rashes related to non-aGVHD conditions. Elafin levels were positively correlated with the severity of skin aGVHD and the overall survival of the patients. Microscopic assessment of skin biopsy samples from patients with aGVHD revealed overexpression of elafin. Therefore, elafin could be used as a biomarker for skin aGVHD.

Biomarkers for detecting gastrointestinal acute graft-versus-host disease: Ferrara et al. (2011) identified REG3α as a plasma biomarker of gastrointestinal GVHD in a multicenter study. REG3α level was three times higher in patients at gastrointestinal GVHD onset than in all other recipients of HSCT (those with non-GVHD gastrointestinal symptoms, non-GVHD enteritis, or skin GVHD). This protein also differentiated between lower gastrointestinal GVHD diarrhea and non-GVHD diarrhea.

Findings from previous studies identified HGF and KRT18 as biomarkers for gastrointestinal GVHD (Luft et al., 2007; Paczesny et al., 2009). Harris et al. (2012) undertook a comparative study to identify the best differential diagnostic indicator among three proteins: REG3α, HGF, and KRT18. All three proteins were significantly elevated in patients with lower gastrointestinal GVHD compared with patients with non-GVHD diarrhea, but REG3α level was best at differentiating between these conditions. Although the level of each of these proteins was elevated in patients with GVHD involving the liver when compared with patients with other causes of hyperbilirubinemia, none of these proteins adequately distinguished between liver GVHD and hyperbilirubinemia.

Taken together, these findings suggest that proteomics can be instrumental in detecting tissue-specific changes from aGVHD and can provide differential diagnosis for skin and gastrointestinal GVHD.

Biomarkers for predicting patient survival: In Ferrara et al.’s (2011) study, REG3α was validated as a diagnostic biomarker of gastrointestinal GVHD and as a biomarker for therapy resistance and mortality. Plasma REG3α level at GVHD onset predicted the treatment response at four weeks, mortality without relapse at one year, and survival at one year (p ≤ 0.001). Multivariate analysis revealed that advanced clinical stage, severe histologic damage, and high REG3α levels at GVHD diagnosis independently predicted mortality without relapse at one year.

ST2 was recently demonstrated to be a significant biomarker for therapy response and death without relapse among patients with aGVHD (Vander Lugt et al., 2013). The patients with high ST2 levels were 2.3 times as likely to develop treatment-resistant GVHD (95% CI [1.5, 3.6]) and 3.7 times as likely to die within six months after therapy (95% CI [2.3, 5.9]) than patients with low ST2 values at the initiation of therapy. ST2 level was also used to stratify patients with aGVHD to predict mortality rates. Patients with low ST2 values had lower mortality rates, and patients with high ST2 values had higher mortality rates without relapse. The authors concluded that the plasma ST2 levels measured at the initiation of therapy for GVHD and during the first month after transplantation significantly predicted treatment-resistant aGVHD and death without relapse.

A clinical trial was undertaken to study the proteomic use for aGVHD management through a multicenter, randomized, four-arm, phase II clinical trial (Levine et al., 2012). Six previously validated proteins (IL-2Rα, TNFR1, HGF, IL-8, elafin, and REG3α) were studied to distinguish between patients with aGVHD who did and did not respond to therapy and to predict survival among patients who received therapy for aGVHD. The
<table>
<thead>
<tr>
<th>Study</th>
<th>Aim</th>
<th>Design</th>
<th>Population</th>
<th>Results</th>
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<tbody>
<tr>
<td>Ferrara et al., 2011</td>
<td>To identify candidate biomarkers for GI GVHD and to evaluate the correlation of REG3α level in patients with GI GVHD, non-GVHD enteritis, and skin GVHD</td>
<td>Prospective study examining the correlation between plasma REG3α concentration and the presence or absence of GI GVHD, as well as the one-year nonrelapse mortality rate</td>
<td>• 20 patients in pilot or discovery set, 871 patients in training set, and 143 patients in validation set</td>
<td>REG3α is a biomarker of GI GVHD.</td>
</tr>
<tr>
<td>Harris et al., 2012</td>
<td>To compare the diagnostic and prognostic use of REG3α, HGF, and KRT18 for lower GI and liver GVHD</td>
<td>Prospective study examining the correlation between plasma REG3α, HGF, and KRT18 concentrations and the presence or absence of GI GVHD, nonresponse to therapy at day 28, and one-year nonrelapse mortality rate</td>
<td>• 826 patients in pilot or training set and 128 patients in validation set</td>
<td>REG3α, HGF, and KRT18 each can be used to predict nonresponse to therapy at day 28. REG3α is the best diagnostic biomarker for GI GVHD.</td>
</tr>
<tr>
<td>Levine et al., 2012</td>
<td>To evaluate the ability of six biomarkers to identify therapy-responsive and nonresponsive patients with GVHD and to predict survival in patients receiving therapy</td>
<td>Multicenter clinical trial examining the correlation between serum IL-2Rα, IL-8, TNFR1, HGF, KRT18, and elafin concentrations and therapy response at day 28 and survival status at day 180</td>
<td>• 180 patients in clinical trial for validation</td>
<td>IL-2Rα, IL-8, TNFR1, HGF, KRT18, and elafin can predict nonresponse to therapy at day 28 and mortality at day 180.</td>
</tr>
<tr>
<td>Paczesny et al., 2009</td>
<td>To identify GVHD biomarkers, validate candidate proteins, and determine the significance of these proteins related to acute GVHD clinical outcomes</td>
<td>Prospective study examining the correlation between proteome and plasma concentrations of IL-2Rα, IL-8, TNFR1, and HGF</td>
<td>• 42 patients in pilot or discovery set, 282 patients in training set, and 142 patients in validation set</td>
<td>The composite of IL-2Rα, IL-8, TNFR1, and HGF can confirm an acute GVHD diagnosis.</td>
</tr>
<tr>
<td>Paczesny et al., 2010</td>
<td>To evaluate candidate plasma biomarkers of skin GVHD and evaluate the prognostic and diagnostic values of elafin for skin GVHD</td>
<td>Prospective study examining the correlation between elafin level and the presence or absence of skin GVHD, GVHD grade, and the risk of death secondary to skin GVHD</td>
<td>• 20 patients in pilot or training set and 492 patients in validation set</td>
<td>Elafin has diagnostic and prognostic value as a biomarker of skin GVHD.</td>
</tr>
<tr>
<td>Vander Lugt et al., 2013</td>
<td>To evaluate the correlation between ST2 level and therapy-resistant GVHD and death</td>
<td>Prospective multicenter trial examining the correlation between plasma ST2 concentration and therapy-resistant GVHD and patient survival</td>
<td>• 20 patients in discovery set, 381 patients in response-to-therapy set, and 673 patients in early stratification set</td>
<td>ST2 is a marker for therapy-resistant GVHD and death without relapse.</td>
</tr>
<tr>
<td>Weissinger et al., 2007</td>
<td>To evaluate the feasibility of differential diagnosis of acute GVHD from other complications following hematopoietic cell transplantation</td>
<td>Prospective study examining the correlation between the presence of 31 polypeptides and prediction of grade 2 or higher GVHD</td>
<td>• 33 patients in pilot or training set and 141 patients in validation set</td>
<td>The 31-polypeptide urine proteome can predict grade 2 or higher acute GVHD.</td>
</tr>
<tr>
<td>Weissinger et al., 2014</td>
<td>To evaluate the predictive value of the proteomic classifier acute GVHD-MS17 in assessing the severity of acute GVHD</td>
<td>Prospective multicenter trial examining the presence of the acute GVHD-MS17 (17-polypeptide) proteomic classifier and the risk for grade 3 or 4 acute GVHD</td>
<td>• 423 patients in validation set</td>
<td>The 17-polypeptide urine proteomic classifier can accurately predict risk for grade 3 or 4 acute GVHD.</td>
</tr>
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GVHD—graft-versus-host disease; GI—gastrointestinal; HGF—hepatocyte growth factor; IL-8—interleukin-8; IL-2Rα—interleukin-2 receptor alpha; KRT—keratin; REG3α—regenerating islet-derived protein 3 alpha; ST2—suppression of tumorigenicity; TNFR1—tumor necrosis factor receptor 1.
level of each of the six serum proteins was measured from samples prospectively collected at three time points (initiation of aGVHD treatment and at two and four weeks into treatment). The level of each protein in samples collected at day 0 was able to predict nonresponse to aGVHD therapy on day 28 after aGVHD onset (odds ratio = 2.98, p = 0.017) and survival status at day 180 (six months) after aGVHD onset (odd ratios = 4.61, p < 0.001). The prospective sampling at multiple treatment centers improved the validity of this study, indicating that proteomics can be a tool for early identification of patients with aGVHD at high or low risk for therapy response and survival.

Implications for Nursing

Oncology nurses are at the forefront of clinical practice driven by scientific, pharmacologic, and clinical advances. Proteomics is one example of an emerging field that has the potential to significantly affect the diagnosis, management, and palliation of aGVHD in recipients of HSCT. In this literature review, eight studies described promising advances in testing of proteins in body fluids with translatability application to clinical practice. Of particular applicability for advanced practice nurses (APNs) is the ability to order proteomic testing for aGVHD, manage the care of patients based on biomarker results, facilitate integration of palliative care based on survival implications of the biomarker results, and educate patients about the presence of aGVHD, including anticipated severity and treatment modalities. APNs may also consult palliative care providers for symptom management. Proteomic testing results can be used to develop individualized care plans for patients with aGVHD, including preparation for limited survival.

All nurses can provide patient education about the types of biomarker testing, their purpose, and the use of test results to guide particular therapeutic approaches. Nurses have a responsibility to support the educational and supportive care needs of patients undergoing proteomic testing. Proteomic testing is highly specialized within the HSCT population, but nurses’ knowledge regarding patient education is critical for these patients.

Recommendations for Future Research

The findings of this review identify key gaps in the literature where nurse clinicians and researchers could make significant contributions. Although the studies reviewed in this article were conducted and published by non-nurse researchers, many opportunities exist for nurses to contribute to the research at the basic science level and in the translation of scientific discoveries to practice. Nurses may also engage in research examining how the use of such testing influences the management of aGVHD and contribute clinical insights when caring for patients with aGVHD. The development of nurse-led research will be critical to further understanding of how the use of proteomic testing for the identification and management of aGVHD affects patients’ quality of life and their experiences. Results of such studies could be helpful in developing evidence-based nursing practices for the management of aGVHD and the support of patients.

Because the use of proteomic testing for this purpose is in its infancy, a dearth of patient educational materials exists regarding the use of this testing to detect aGVHD. Nurses could contribute significant clinical knowledge to the development of patient educational resources regarding how the testing samples are collected, what testing is able to detect, and how results may be used to manage aGVHD.

Conclusion

Proteomics is a revolutionary field that employs advanced technologies to identify and measure the levels of proteins that best represent the pathophysiology of aGVHD. Familiarity with and ability to educate patients about the clinical relevance of advances in proteomic biomarkers is imperative for oncology nurses and APNs engaged in caring for these patients. Proteomics offers great promise to identify prognostic indicators for overall health, optimal outcomes, and mortality. Nurse clinicians and researchers could make significant contributions to the translation of proteomics to the clinical management of aGVHD, research on, and patient education about aGVHD.

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References


Implications for Practice

- Use proteomics, an emerging noninvasive tool, with existing tests to determine the overall prognosis and coordinate the care of patients with acute graft-versus-host disease (aGVHD).
- Educate patients with aGVHD about advances in proteomics technology and the clinical relevance of proteomics results.
- Design, modify, and deliver individualized plans of care using proteomics for better management of aGVHD or palliation of symptoms in patients with aGVHD.


