Proteomic screening applied to the diagnosis of chronic graft-versus-host-disease

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Abstract:
Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a curative treatment for many hematologic malignancies or hematopoietic dysfunction syndromes, but the application is still limited due to major complications, such as severe graft versus host disease (GvHD) and infectious complications. Diagnosis chronic GvHD is based on clinical features and biopsies, a non-invasive, unbiased laboratory test does not exist. We used the urine collected from 20 patients (10 with limited cGvHD, 10 with extensive cGvHD) to establish a proteomic pattern that allowed the diagnosis of cGvHD development and tested the resulting set of polypeptide markers (27 differentially excreted peptides) on more than 200 patients prospectively and blindly for the correct classification of cGvHD samples. The majority of the patients included were transplanted for hematological malignancies (n=209), 6 for hematopoietic failure syndromes. Conditioning regimens included dose reduced conditioning regimens (FLAMSA and ClarAc for the majority of the patients of MHH), as well as standard conditioning regimens (TBI+Cy or Busulfan+Cy) for about 35% of the patients, with GvHD-prophylaxis including cyclosporine A and mycophenolate (MMF) or metothrexate (MTX) as appropriate. Eighty percent of the patients received ATG (antithymocyte globulin) prior to HSCT. A peptide pattern of 27 peptides, differentiating from acute to chronic GvHD was developed. Controls were patients at least 10 days post HSCT, with no GvHD in the history, no infections and without relapse at the time of sampling. Prospective and blinded evaluation of the patients revealed the correct classification of patients developing GvHD with a sensitivity of 85% and specificity of 95%. Further evaluation of the cGvHD patterns specific for particular organs manifests of cGvHD are currently ongoing. Interestingly, the cGvHD pattern seems to be predictive for GvHD developing post DLI, while the aGvHD-specific proteomic pattern only predicts GvHD of the intestine, which may be more similar to "late acute GvHD".

Methods:
Samples are collected and stored immediately on -20°C. Prior to measurement, samples are prepared by removing salt and other confounding material (molecules with a mass greater than 20 kDa). Then samples are loaded onto the capillary electrophoresis (CE) and are directly sprayed into the mass spectrometer (MS). Spectra are generated every 3 min and are stored in the data base. Application of Mosaiques-Visu allows depiction of the data as 3-dimensional blots as shown in Figure 2.

Results:
Biomarker pattern specific for complications after HSCT (like cGvHD, and others) are established. Figure 3 shows the differences between patients with cGvHD, controls after day +160 (no infection, no relapse, no GvHD) and patients with aGvHD, as another control for cGvHD-patients. The 27 selected polypeptides are discriminatory between the different patients and allow distinction of tolerant patients with no complications. Application of the pattern to patients screened routinely in Hannover allows diagnosis of GvHD with a specificity of 88% and a sensitivity of 85%.

Conclusions and outlook:
- The cGvHD-specific pattern is currently used for diagnosis of developing cGvHD in our patients.
- The pattern can also be used to detect GvHD developing after donor leukocyte transfusion (DLT) and yields better correct classification than the acute GvHD-specific proteomic pattern.

Table 1: Training set used to establish the proteomic pattern for early diagnosis of cGvHD

<table>
<thead>
<tr>
<th>Age at HSCT</th>
<th>DSS/AR</th>
<th>PR</th>
<th>TX/PR</th>
<th>Remission</th>
<th>CDG grade</th>
<th>day pre HSCT</th>
<th>day post HSCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-29</td>
<td>0-17</td>
<td>0-24</td>
<td>0-32</td>
<td>0-41</td>
<td>0-54</td>
<td>0-67</td>
<td>0-80</td>
</tr>
</tbody>
</table>

Table 1 shows the training set used to establish the proteomic pattern for early diagnosis of cGvHD.

Ten patients with limited and 10 with extensive cGvHD were chosen to establish the pattern. The majority of the patients had acute leukemia, mainly AML and were transplanted from MUD. These patients are representative for patients transplanted in our department.

Definition of polypeptides specific for cGvHD (Figure 2)
The application of Mosaiques-Visu allows the generation of protein plots (A) and peak lists summarizing all information obtained for mass, charge and signal intensity, these data are then converted into raw mass by deconvolution and (mass kB) and signal intensity (which is shown as a color code ranging from blue to white) are stored in the data base and this allows compilation of individual data of all patients with or without cGvHD to form 2 different groups (B). Each individual pattern obtained from urine with screened automatically by the software (MosaCluster) for the identification of biomarkers for clinical diagnosis.

Figure 1: On-line coupling of capillary electrophoresis and mass spectrometer

Figure 2: Data processing and compilation of data
A: Raw data evaluation and further processing
B: Compilation of data

Figure 3: Proteomic pattern for cGvHD

Figure 4: Classification factor CF over the time course after HSCT

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