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Gene Expression Profiling To Predict Viridans Group Streptococcal and Invasive Fungal Infection in Pediatric Acute Myeloid Leukemia: A Brief Report from the Children's Oncology Group

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Acute myeloid leukemia (AML) is the second most common leukemia in childhood, requiring one of the most intensive chemotherapeutic regimens used in pediatric oncology. While long-term cure rates have improved with the use of intensive chemotherapy, infection is a significant source of morbidity and mortality for pediatric AML patients.[1–3] Viridans

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Conflicts of Interest

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group streptococcal (VGS) bloodstream infection (BSI) and invasive fungal infection (IFI) are major causes of morbidity and mortality in pediatric AML.[1, 4–6] Genome-wide expression profiling reflects the dynamic influence of environment on transcriptional activity, and has led to the identification of characteristic expression “signatures” of various infectious agents in actively infected patients.[7–9] We investigated the novel application of expression profiling to identify increased host susceptibility to VGS BSI and IFI, hypothesizing that identification of differential constitutional expression of immune pathways prior to the occurrence of infectious events might permit the prediction and prevention of VGS and IFI complications in pediatric AML.

A case-control pilot study was conducted using existing banked bone marrow specimens from the Children’s Oncology Group (COG) AAML0531 group-wide phase III study. AAML0531 was a COG Phase III randomized controlled trial that investigated the use of Gemtuzumab ozogamicin (GMTZ) combined with conventional therapy for *de novo* AML in patients between the ages of 1 month to 30 years. The pilot study protocol was not considered human subjects research and was exempt from review by the IRB at The Children’s Hospital of Philadelphia. Study participants with available bone marrow specimens at end of Induction I or II were eligible for inclusion. Only white patients without Trisomy 21 were included. Bone marrow samples showing tumor cell contamination or not meeting RNA quality standards were excluded. VGS cases were defined as patients who developed their first VGS BSI after bone marrow specimen procurement, during Induction or Intensification cycles. IFI cases were defined as patients with microbiological evidence of fungal infection from sterile sites and were identified similarly to VGS cases. Controls were defined as patients who developed neither VGS BSI nor IFI during Induction or Intensification phases. Bone marrow specimens were shipped overnight at room temperature to the AML reference laboratory to maximize viability. Total RNA was isolated using Qiagen RNeasy®. Microarray services were provided by the Penn Profiling Facility. All protocols were conducted as described in the Ambion® WT Expression Kit for Affymetrix® GeneChip® Whole Transcript (WT) Expression Arrays manual. Baseline characteristics of case and control groups were compared by Kruskal Wallis equality of proportions rank test for continuous variables and Fisher’s exact test for categorical variables, using STATA statistical software v. 12.0 (College Station, TX). Expression analysis was performed by the Bioinformatics Group of the Penn Molecular Profiling Facility. Robust multiple-array average normalization was applied, and unsupervised analysis was performed using principal components analysis. Class comparison between cases and controls was then performed using one-way ANOVA, with multiple testing correction.

Eighteen VGS cases, 8 IFI cases, and 22 controls met inclusion and exclusion criteria and were included for microarray processing and analysis. The 18 VGS cases represented a total of 32 episodes of VGS bacteremia. There was one IFI event for each IFI case. Five of 8 IFI cases also had VGS infection during the study period. 50% of VGS cases, 50% of IFI cases, and 65% of controls were males ($p=0.46$). The median age at diagnosis of the VGS cases, IFI cases, and control group was 7.7 years, 13.4 years, and 10.4 years, respectively ($p=0.49$). All bone marrow specimens from controls and IFI cases were obtained at end of Induction I. Sixteen of 18 VGS case specimens were obtained at end of Induction I, and 2 of 18 VGS

case specimens were obtained at end of Induction II ($p=0.29$). Median RNA concentration in cases and controls differed significantly ($p=0.01$), with VGS and IFI cases having lower median RNA concentrations than that of controls. Median 260:280 in cases and controls did not differ significantly ($p=0.33$). Principal components analysis did not reveal significant differences among the three groups. Class comparison using one-way ANOVA did not reveal genes with a >1.5 fold difference in expression between groups, and no gene expression patterns were found to be predictive of the development of VGS or IFI events.

In this case-control pilot study, we were unable to show any significant differences in pre-infection gene expression that would allow prediction of VGS or IFI events. This study had several limitations that may have contributed to the negative result. Delays inherent in shipment of bone marrow samples and resulting suboptimal storage conditions prior to arrival at the COG AML laboratory may have resulted in degraded RNA transcripts that confounded the study results.[10] The diverse cell type population within the bone marrow could create significant background signal to mask any meaningful expression pattern produced by a specific cell lineage comprising a minority of the cell population. Future work may benefit from sorting by cell types to allow comparison of gene expression profiles within homogeneous cell populations. Last, the small number of subjects meeting study inclusion criteria resulted in limited statistical power and ability to control for potential confounders.

In summary, we performed genome-wide expression analysis of bone marrow specimens from subjects with pediatric AML to identify a gene expression profile predictive of the development of VGS and IFI complications during chemotherapy. We were unable to distinguish any differences in expression patterns between cases and controls. Further studies are needed to establish the potential for gene expression profiling as a predictive tool for infectious outcomes in pediatric AML patients.

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