Prevalence of Chromosomally Integrated Human Herpesvirus 6 in Patients with Human Herpesvirus 6—Central Nervous System Dysfunction

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INTRODUCTION

Human herpesvirus 6 (HHV-6) has a unique ability to integrate into chromosomal telomeres of infected cells [1]. When this occurs in germ cells, Mendelian inheritance results in offspring with chromosomally integrated (ci) HHV-6 in every nucleated cell. This condition is present in ~1% of people [1], can be the source of viral reactivation [2,3], and has been implicated in HHV-6 central nervous system (CNS) disease [4,5]. To explore whether patients with ciHHV-6 have increased risk for HHV-6—associated disease, we tested the prevalence of ciHHV-6 in allogeneic hematopoietic cell transplantation (HCT) recipients with HHV-6 CNS dysfunction.

We identified 37 allogeneic HCT recipients at our center with HHV-6 CNS dysfunction as previously described [6]. We tested archived samples from donor-recipient pairs for ciHHV-6 using a novel method to detect HHV-6 and human ribonuclease P (RPP30, a reference gene for cell count) DNA by droplet digital PCR (Figure 1) [7]. CIHHV-6 was ruled out if the ratio of HHV-6 DNA to cell genome equivalents (2 RPP30/cell) fell outside the range of 1 ± 0.07.

CIHHV-6 species A was detected in 1 pre-HCT patient sample (prevalence 2.7%; 95% confidence interval, 0.7% to 14.5%) from a 53-year-old man who developed findings consistent with HHV-6 encephalitis after a myeloablative matched, related donor HCT. He initially developed hallucinations and agitation on day 11 after HCT, 1 day after starting methylprednisolone 2 mg/kg/day for skin rash. Cerebrospinal fluid (CSF) on day +12 had 2 lymphocytes, 4 red blood cells, and protein 43 mg/dL. Bacterial, mycobacterial, and fungal stains and cultures were negative, as was PCR for other herpesviruses. Semiquantitative PCR for HHV-6 DNA was positive with 2500 copies/mL. The patient was started on foscarnet 90 mg/kg i.v every 12 hours on day +14 for HHV-6 encephalitis.

The patient had initial improvement in mental status followed by progressive encephalopathy. He engrafted on day +17 and was weaned off steroids by day +23. Repeat CSF testing on day +26 had 2 lymphocytes, 54 red blood cells, and 2500 copies/ml HHV-6 DNA by semiquantitative PCR. Additional testing as above was negative. Foscarnet was discontinued on day +29 due to acute kidney injury. Chimerism and flow cytometry studies of bone marrow and blood on day +29 were of donor origin, and PCR for HHV-6 in bone marrow was negative at that time. Brain magnetic resonance imaging was negative on day +33. The patient developed hypoxia on day +38 attributed to aspiration in the setting of obtundation and died on day +40 because of progressive pulmonary disease. An autopsy specimen revealed possible increased brain microglia and perivascular lymphocyte cuffing, as well as scattered focal hippocampal neuronal dropout; although nonspecific, this could be consistent with viral encephalitis.

Retrospective testing of multiple archived serum samples with droplet digital PCR detected HHV-6 DNA with decreasing HHV-6/cellular DNA ratios of .5 to .02 (days 5 through 39). This could be consistent with allograft replacement of recipient ciHHV-6 hematopoietic cells or treatment of HHV-6 reactivation with foscarnet. Sanger sequencing of a 900 base pair region of the HHV-6 envelope glycoprotein B gene in a pre-HCT cell sample and post-HCT serum sample (day +39) revealed complete homology but divergence from 14 other strains [8] (Figure 2). A fresh-frozen brain section of the inferior temporal lobe had an HHV-6/cellular DNA ratio of 1.0, consistent with ciHHV-6 but without evidence of additional HHV-6 replication. Testing of formalin-fixed, paraffin-embedded samples from the medial temporal lobes (the predominant site of HHV-6 CNS disease)
yielded inconclusive results because of poor preservation. RNA testing and viral culture could not be performed on archived specimens.

This is the first epidemiologic study of the prevalence of ciHHV-6 in patients with HHV-6-associated CNS dysfunction. Convincing evidence of HHV-6 reactivation and pathogenicity from ciHHV-6 cell lines is well-described [2-5]. We did not identify a clear overrepresentation of ciHHV-6 in this cohort of 37 patients compared with population-based studies [1], but the confidence interval was wide and suggests an incidence as high as 14.5%. We also describe the first case of a patient with ciHHV-6A and HHV-6A reactivation as a possible cause of post-HCT encephalitis.

Figure 1. Flow diagram for chromosomally integrated HHV-6 testing. Archived pre-HCT patient and donor beta-lymphoblastoid cell lines were the primary source for ciHHV-6 testing. When unavailable, other appropriate specimens (serum or bone marrow) were used as surrogates. *Surrogate for donor sample in cases that were missing pre-HCT donor sample. These were obtained after engraftment. One patient had a HHV-6/cellular DNA ratio significantly >1, making it impossible to rule out "reactivation. De novo HHV-6 infection was unlikely given sequence homology between pre and post-HCT HHV-6 strains. Recipient hematopoietic cells with ciHHV-6A would be an unlikely source of persistent HHV-6A DNA in serum or CSF samples after a myeloablative HCT, especially given evidence of full donor chimerism and absence of HHV-6 DNA detection in the day +29 bone marrow biopsy. Unfortunately, RNA testing and viral culture of saved specimens were not possible because of prior processing and extended storage.

In conclusion, we did not find evidence of ciHHV-6A enrichment in a cohort of HCT patients with HHV-6 CNS dysfunction compared with the general population, but larger studies are needed to comprehensively determine the true incidence and prevalence of reactivation in high-risk patients. Our findings begin to address the call to reevaluate transplantation practices in the setting of ciHHV-6 [9] and underscore the need for large, multicenter collaborations to determine the impact of ciHHV-6 on patient outcomes.

Figure 2. Phylogenetic analysis of HHV-6A glycoprotein B (gB) gene sequences from pre and post-HCT samples of the described patient with ciHHV-6A compared with ciHHV-6A from 4 patients at our center, the Hector-2 cell line (Bioworld Consulting Laboratories, Mt. Airy, MD), and 9 published sequences [8]. This figure demonstrates that the sequence of an ~900 base pair region of the HHV-6A gB gene in the discussed patient’s pre-HCT beta-lymphoblastoid cell line and post-HCT (day +30) serum sample were identical and diverged from 14 other available sequences. Bar indicates number of nucleotide changes per 100 sites. The gB gene sequences of 9 HHV-6A primary or laboratory-adapted isolates were obtained from Dr Henri Agut (Paris, France), and the geographical origins were as follows: GS, United States; U1102, Uganda; SIE, Côte d’Ivoire; TAN, Congo; and 1120, E540, 132, 1116, 719, and, p523, France. Nucleotide sequences were submitted to GenBank and assigned the following accession numbers: Hector-2control, KM592979; Pre-HCT_B-lymphoblastoid_cells, KM592980; Post-HCT_serum, KM592981; UWciHHV6_1, KM592982; UWciHHV6_2, KM592983; UWciHHV6_3, KM592984; UWciHHV6_4, KM592985. The tree was constructed from the comparison of gB gene nucleotide sequences using the free Phylogeny.fr webservice, available at http://www.phylogeny.fr/version2/cgi/index.cgi.

Although HHV-6A DNA detected in post-HCT samples may have represented cells with ciHHV-6A rather than active replication, and alternative causes of encephalitis were possible, we think the findings are best explained by HHV-6A reactivation. De novo HHV-6 infection was unlikely given sequence homology between pre and post-HCT HHV-6 strains. Recipient hematopoietic cells with ciHHV-6A would be an unlikely source of persistent HHV-6A DNA in serum or CSF samples after a myeloablative HCT, especially given evidence of full donor chimerism and absence of HHV-6 DNA detection in the day +29 bone marrow biopsy. Unfortunately, RNA testing and viral culture of saved specimens were not possible because of prior processing and extended storage.

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REFERENCES


Number of Courses of Induction Therapy Independently Predicts Outcome after Allogeneic Transplantation for Acute Myeloid Leukemia in First Morphological Remission

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ABSTRACT

Whether the number of chemotherapy cycles required to obtain a first morphological remission affects prognosis of patients with acute myeloid leukemia (AML) remains controversial. To clarify how achievement of early remission might influence outcome of allogeneic hematopoietic cell transplantation (HCT), we studied 220 consecutive adults with AML in first morphological remission who underwent transplantation after myeloablative or nonmyeloablative conditioning to investigate how the number of standard- or high-dose induction courses required to achieve remission impacted post-HCT outcome. Three-year estimates of overall survival were 65% (95% confidence interval [CI] 56% to 73%), 56% (95% CI, 43% to 67%), and 23% (95% CI, 6% to 46%) for patients requiring 1 course, 2 courses, or >2 courses of induction therapy; corresponding relapse estimates were 24% (95% CI, 17% to 31%), 43% (95% CI, 31% to 55%), and 58% (95% CI, 30% to 78%), respectively. After covariate adjustment (minimal residual disease status, conditioning, age, cytogenetic disease risk, type of consolidation chemotherapy, pre-HCT karyotype, and pre-HCT peripheral blood count recovery), the hazard ratios for 2 or >2 induction courses versus 1 induction were 1.16 (95% CI, .73 to 1.85, P = .53) and 2.63 (95% CI, 1.24 to 5.57, P = .011) for overall mortality, and 2.10 (95% CI, 1.27 to 3.48, P = .004) and 3.32 (95% CI, 1.42 to 7.78, P = .006), respectively, for relapse. These findings indicate that the number of induction courses required to achieve morphological remission in AML adds prognostic information for post-HCT outcome that is independent of other prognostic factors.

INTRODUCTION

For many patients with acute myeloid leukemia (AML) in first remission, allogeneic hematopoietic cell transplantation (HCT) is an effective consolidation therapy. Still, even in the absence of morphologically detectable disease at the time of transplantation, relapse remains a major cause of treatment failure [1,2], although it is widely appreciated that the risk of disease recurrence varies considerably among patients. Hence, there has been interest in understanding pretransplantation factors that could serve as predictors of adverse post-HCT outcome to inform patients accurately about likely treatment outcomes and to develop risk-stratified transplantation regimens.

Recent attention has focused on the role of pretransplantation minimal residual disease (MRD) as indicator of increased risk of relapse after allogeneic HCT for patients...