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Past, present, and future perspectives on the diagnosis of Roseolovirus infections

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Diagnosis of Roseolovirus infections mandates careful selection of patients, samples, and testing methods. We review advances in the field and highlight research priorities. Quantitative (q)PCR can accurately identify and distinguish between human herpesvirus 6 (HHV-6) species A and B. Whether screening of high-risk patients improves outcomes is unclear. Chromosomally integrated (ci)HHV-6 confounds test interpretation but can be ruled out with digital PCR. Reverse transcription qPCR may be a more specific and clinically applicable test for actively replicating Roseoloviruses, particularly among patients with ciHHV-6. Interpretation of Roseolovirus test results faces many challenges. However, careful application of refined and emerging diagnostic techniques will allow for increasingly accurate diagnosis of clinically significant infections and disease associations.

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Introduction

The *Roseolovirus* genus of the betaherpesvirus subfamily is composed of three enveloped, double-stranded DNA viruses: human herpesvirus (HHV-) 6A, HHV-6B, and HHV-7 [1]. These viruses share many properties that include virion structure, genomic sequence, and epidemiology but have important molecular and biologic differences [2•]. Like other human herpesviruses, infection with Roseoloviruses occurs early in life, results in chronic viral latency in diverse cell types, and affects the population at large. These characteristics complicate diagnostic efforts to determine whether Roseoloviruses are causative in many implicated diseases. Additional confusion has developed due to the unique ability of HHV-6A and HHV-6B to integrate into chromosomal telomeres of infected cells [3] as reviewed in this issue by Kaufer et al. When this occurs in a germ cell, vertical transmission of inherited chromosomally integrated (ci)HHV-6 results in offspring with latent HHV-6 DNA in every nucleated cell of their body. To further complicate matters, there is evidence that biologically active HHV-6 can reactivate in individuals with inherited ciHHV-6 and cause disease [4,5••,6]. This review highlights important advances in the diagnosis of Roseolovirus infections and provides guidance for application of current and developing diagnostic methods.

Who to test

Roseoloviruses have been variably associated with many diseases in diverse patient groups. Primary HHV-6B infection occurs in the majority of children by two years of age and usually results in a typical presentation of exanthem subitum (roseola) with mild symptoms including fever and rash [7]. HHV-6A and HHV-7 primary infection have epidemiologic differences in comparison to HHV-6B but also appear to occur in childhood with similar presentations [8–10]. Serious complications are infrequent, although primary infection with Roseoloviruses leads to significant healthcare utilization [7], and HHV-6B or HHV-7 have been associated with approximately one-third of cases of febrile status epilepticus [11]. Although testing for Roseoloviruses in the setting of typical exanthem subitum is generally not indicated, quick and accurate diagnosis could play a role in stemming antimicrobial overuse, minimizing unnecessary hospitalization, informing potential utility of selective treatment, and advancing understanding of the clinical impact of primary infection (Table 1). Primary infections are reviewed in detail in this section by Tesini et al.

The majority of known complications due to Roseoloviruses result from HHV-6B reactivation in immunocompromised patients, specifically those undergoing hematopoietic cell (HCT) or solid organ transplantation (SOT) as reviewed in this issue by Hill and Zerr [12]. Selective testing is important among these patients (Table 1). HHV-6B and HHV-7 reactivation after HCT or SOT occurs in 40–50% of patients, whereas HHV-6A reactivation is infrequent [13–15]. HHV-6A and HHV-7 do not appear to be important pathogens in these patients. However, HHV-6B has been associated

Table 1

Summary of key diagnostic considerations for clinical testing of HHV-6B[a]

Patient selection	**Comments**	
• Primary infection	• Rarely results in significant morbidity, routine testing not indicated but may stem inappropriate use of healthcare resources	
• Reactivation after HCT	• Frequent finding with multiple associated complications, targeted testing indicated	
• Other	• Selective testing should be considered in other immunocompromised and immunocompetent patients with HHV-6B-associated complications	
Test selection	**Strengths**	**Weaknesses**
• Quantitative PCR	• Sensitive, quantitative, efficient, distinguishes species	• Not standardized, detects latent virus
• Digital PCR	• Better accuracy and precision, useful for detecting ciHHV-6	• More expensive and labor intensive, detects latent virus
• Reverse transcription PCR	• Positive results represent active replication	• More expensive and labor intensive
Sample selection	**Strengths**	**Weaknesses**
• Whole blood, serum, plasma	• Easy to access and process	• May contain latent virus, not a perfect surrogate for end-organ disease
• Tissue	• Appropriate testing provides stronger evidence for causality	• May contain latent virus, difficult to obtain
• Other (e.g. CSF, BALF)	• Better surrogate for end-organ disease than blood fractions	• May contain latent virus, difficult to obtain

HHV-6, human herpesvirus 6; HCT, hematopoietic cell transplantation; PCR, polymerase chain reaction; ciHHV-6, inherited chromosomally integrated HHV-6; CSF, cerebrospinal fluid; BALF, bronchoalveolar lavage fluid.

[a] Testing for HHV-6A or HHV-7 should be considered on a case-by-case basis, as there is little evidence to support any definitive disease association for either virus.

with many complications in HCT recipients, most notably central nervous system (CNS) disease [13,16,17]. Accordingly, it is reasonable to test transplant recipients for HHV-6B in the setting of any end-organ disease and particularly those with encephalopathy. Although readily available antiviral medications can abrogate viral reactivation when used as a preventive measure, this has not resulted in statistically significant improvement in associated outcomes in a few small studies [18–20]. Whether routine monitoring for HHV-6 in transplant recipients can improve outcomes remains unclear [15].

Testing for Roseoloviruses in other patient groups with findings suggestive of herpesvirus pathogenicity and an otherwise negative workup should be considered (Table 1). Ultimately, testing should be ordered judiciously in all settings, and results must be interpreted in the context of the clinical scenario, sample source, and possibility of inherited ciHHV-6.

Clinical testing and specimen selection

We again underscore that test and specimen selection for Roseolovirus testing should be guided by the clinical context. Direct detection of Roseoloviruses by culture is considered the gold-standard test for active infection, but this method is labor intensive, slow, and unsuitable for routine clinical use [1]. Indirect methods to detect an immunological response have limited utility for clinical use [21]. Numerous serologic assays have been described, including indirect fluorescent-antibody and enzyme-linked immunosorbent assay. IgM testing is not useful for clinical diagnosis of primary infection [22], and most assays are unable to discriminate prior infections with HHV-6A from HHV-6B, although a recently described assay appears to enable variant-specific serologic testing [23]. Current antigenemia tests are inadequate for distinguishing low-level viral reactivation from clinically relevant infection [24,25]. Immunohistochemistry and in situ hybridization are rarely used clinically due to limited sensitivity and slow turn-around time. Selective application of DNA testing by polymerase chain reaction (PCR) assay, however, meets important criteria for clinical use: it is sensitive, quantitative, and precise; it can distinguish between species; and it can be efficiently performed [26•]. Accordingly, PCR for Roseolovirus DNA has become the mainstay of clinical diagnostics. We focus our discussion on diagnostic techniques for HHV-6 species (Table 1).

A variety of qPCR assays for measuring HHV-6 DNA viral load are in clinical use in laboratories across the world [26•,27,28]. Well-validated assays target conserved regions of the HHV-6 genome, and some are able to differentiate HHV-6A and HHV-6B. Early PCR assays that used qualitative, nested approaches had high sensitivity but were prone to false-positive results. Quantitative real-time PCR (qPCR) has emerged as the most sensitive and rapid method available for clinical diagnosis of Roseolovirus infection or reactivation. However, inter-lab quantitative agreement for HHV-6 viral load is poor [27,29], and there is currently no international standard available for HHV-6B or HHV-6A. These factors complicate implementation of commutable assays with clinically meaningful viral load thresholds to validate research findings and guide treatment decisions [30]. The development of an international standard, such as the one for