

Clinical Spectrum and Impact of Enterovirus Detection and Strain Typing by CSF Metagenomic Next-Generation Sequencing: A Multicenter Case Series

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Background

- Enterovirus (EV) is a common cause of CNS infection
- Conventional testing (e.g., PCR-based assays) detects EV but lacks strain-level resolution
- EV subtype may influence clinical severity, epidemiology, and therapeutic considerations

Objectives

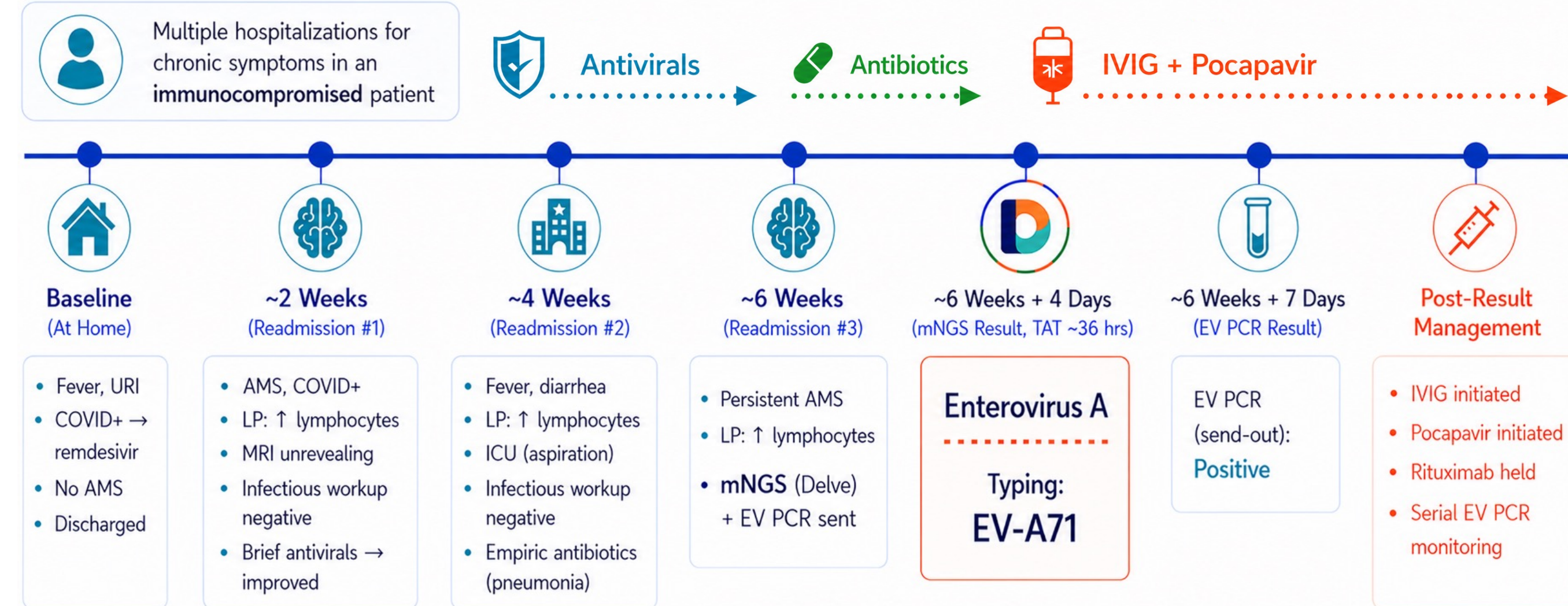
- Evaluate CSF mNGS for enterovirus detection and strain typing in CNS infection
- Assess how strain-level resolution informs prognostic assessment and therapeutic decision-making

Methods

- Multicenter case series across 4 U.S. centers
- Cases identified through clinical consultation program
- Clinical data obtained via retrospective chart review
- CSF mNGS:
 - ≥10 million reads per sample, analyzed against a curated microbial database
 - Enterovirus strain typing

Chronic EV A71 in an Immunocompromised Patient

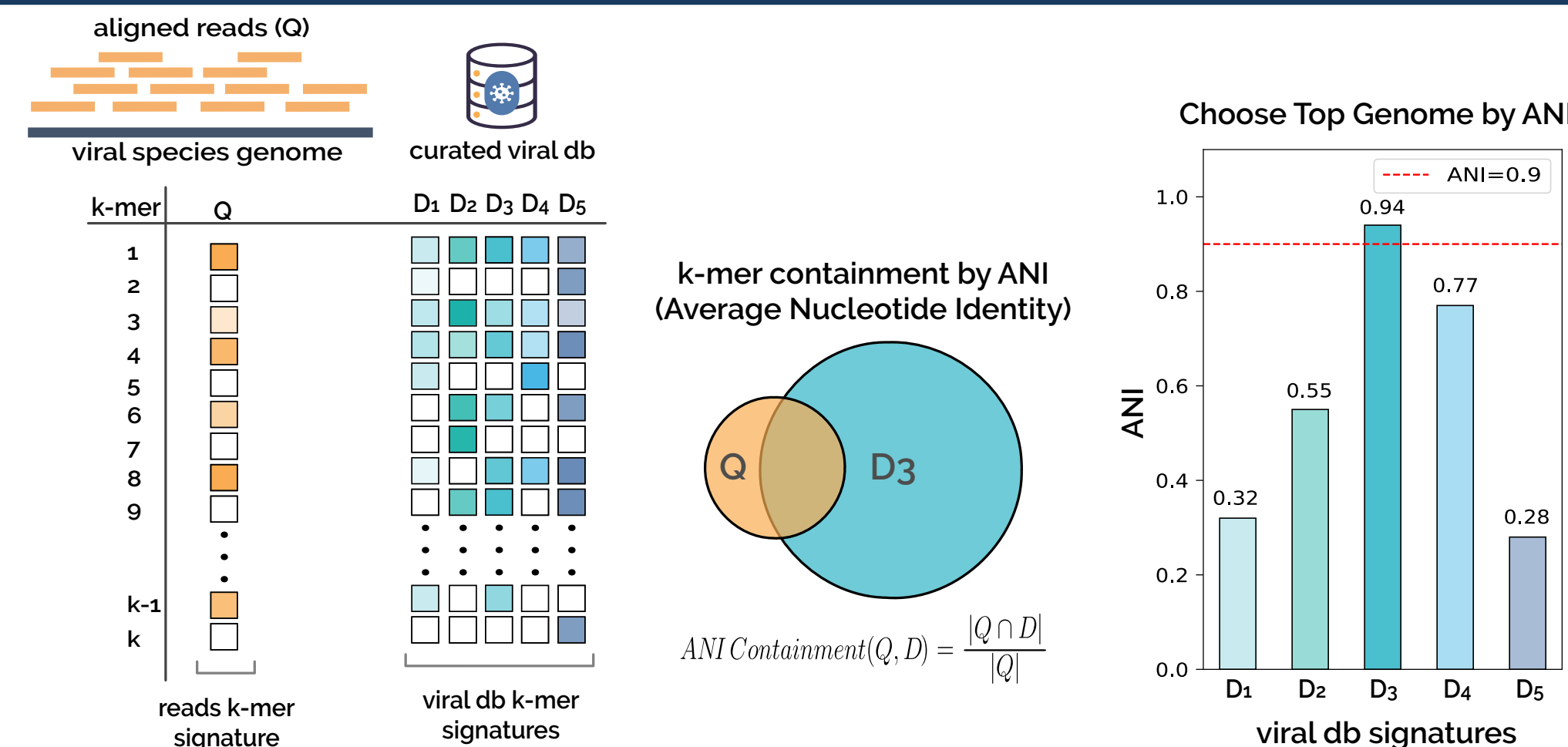
60s F with altered mental status | Follicular lymphoma on anti-CD20 therapy (rituximab)



Case Detections

Patient	EV Subtype	Reads	Coverage	Clinical Impact
Adult – Lymphoma	Enterovirus A71	121	51%	IVIG + poczapavir; rituximab held
Adult – MS	Coxsackievirus A16	16	18%	IVIG + poczapavir; rituximab held
Adult – SLE	Coxsackievirus A16	42	31%	Supportive management
Adult – MS	Coxsackievirus B3	63	39%	Supportive management
Adult – MS	Coxsackievirus B1	31	12%	Supportive management
Infant	Echovirus E11	364	98%	Supportive management
Infant	Echovirus E11	264	95%	Supportive management

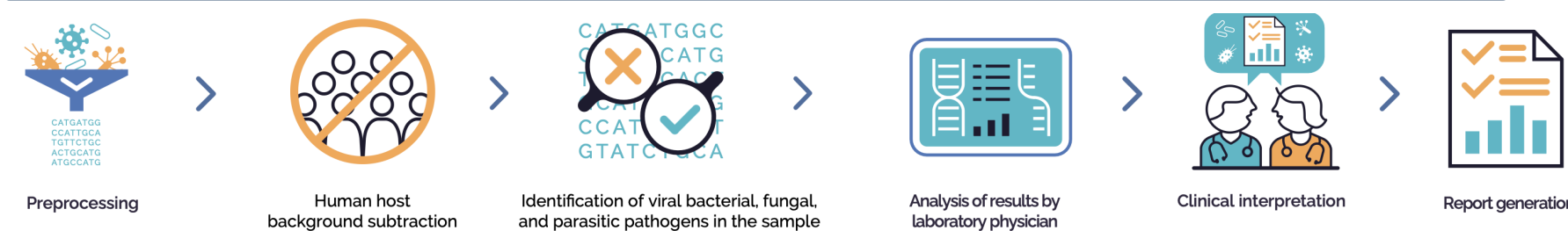
Viral Subtyping



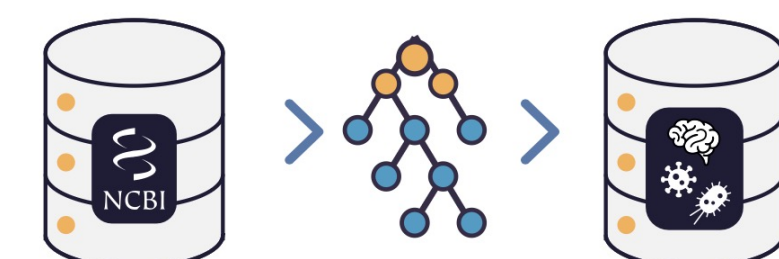
Clinical Implications

- EV CNS infection in anti-CD20-treated patients can be severe, prolonged, or atypical
- Presentations include meningoencephalitis, encephalopathy, acute flaccid paralysis/myelitis, and infant meningitis
- Conventional testing may detect EV but usually lacks strain-level resolution
- Strain typing may inform prognosis, surveillance, and therapy selection, including IVIG and investigational antivirals such as poczapavir

Pathogen Detection Through mNGS



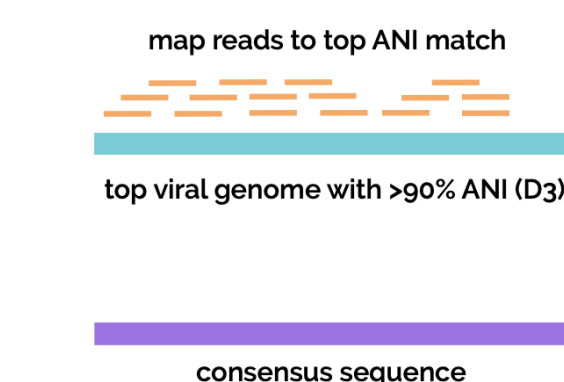
Curated Pathogen Database



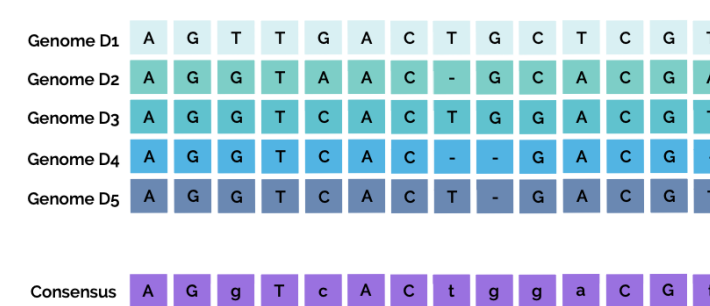
- Filtered from NCBI NT for CNS-relevant organisms
- Deduplicated, high-quality references
- >68000 organisms

Bacteria >26,000 species	Viruses >10,000 species
Fungi >16,000 species	Parasites >15,000 species

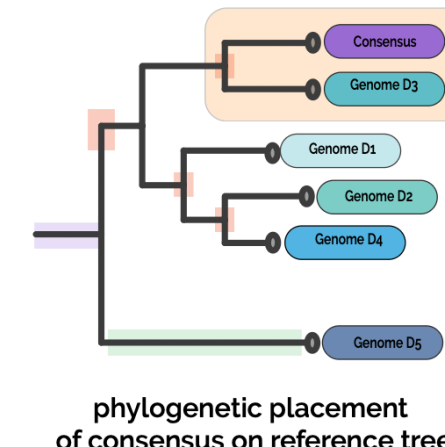
Templated Assembly of Reads



Multiple Sequence Alignment (MSA)



Phylogenetic Placement



Viral reads are converted to k-mer signatures and matched to a curated reference database to identify the closest subtype and assess confidence. High-confidence matches are then used for templated assembly and phylogenetic placement using MSA.

Conclusions

- CSF mNGS enabled timely EV detection and strain typing in CNS infection
- Identified clinically relevant strain diversity, contributing to management decisions in selected high-risk patients
- May be particularly valuable in immunocompromised hosts, unexplained or persistent CNS syndromes