Fondazione IRCCS Policlinico San Matteo

Sistema Socio Sanitario

Regione Lombardia

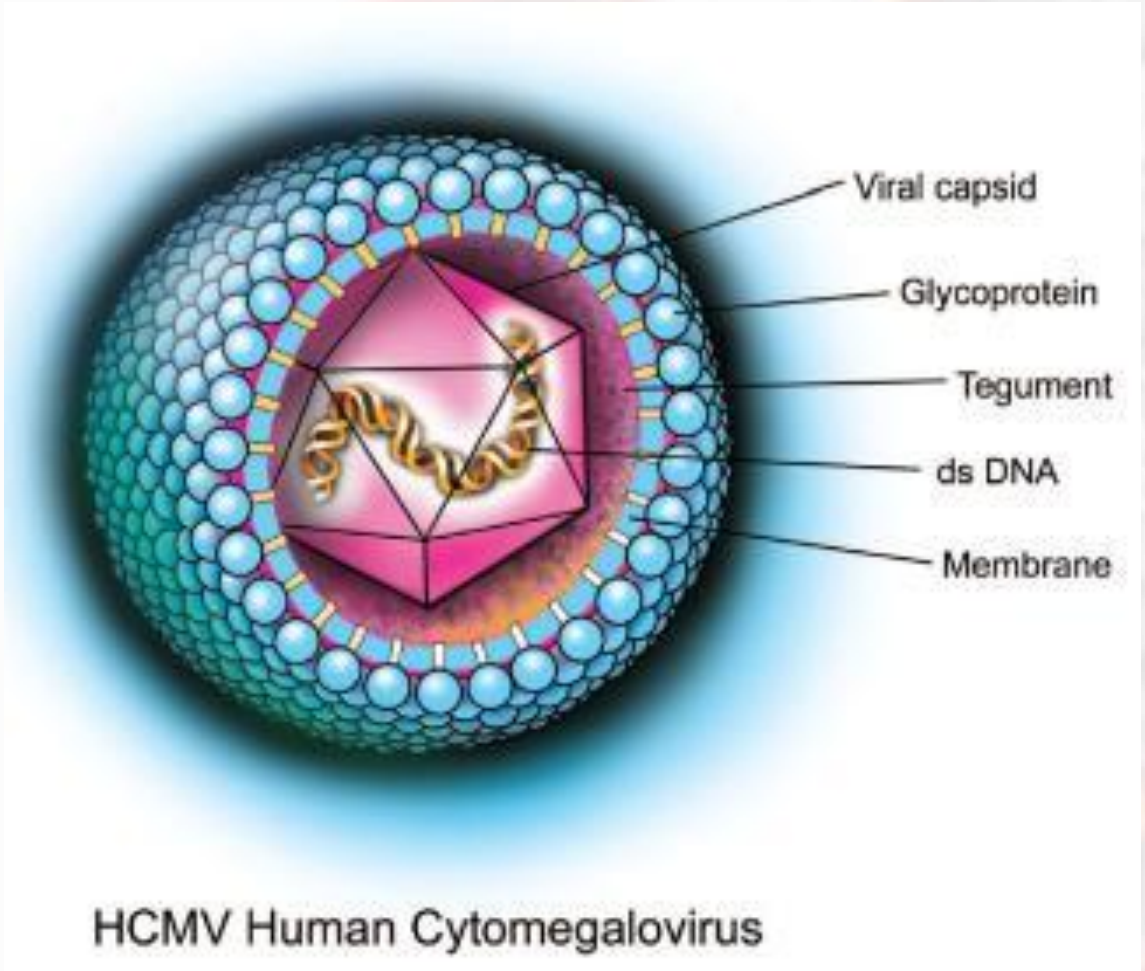
CITOMEGALOVIRUS UMANO

CARATTERISTICHE DEL VIRUS E METODI DIAGNOSTICI

Daniele Lilleri

HERPESVIRIDAE

***alfa*-herpesvirus:**	**Herpes Simplex Virus 1 (HHV-1)**
	Herpes Simplex Virus 2 (HHV-2)
	Varicella Zoster Virus (HHV-3)
***beta*-herpesvirus:**	**Human Cytomegalovirus (HHV-5)**
	Human Herpes Virus 6A (HHV-6A)
	Human Herpes Virus 6B (HHV-6B)
	Human Herpes Virus 7 (HHV-7)
***gamma*-herpesvirus:**	**Epstein-Barr Virus (HHV-4)**
	Human Herpes Virus 8 (HHV-8)



HCMV Human Cytomegalovirus

100 anni di storia

- **1921.** Goodpasture e Talbow usano per la prima volta il termine «**citomegalia»** per descrivere cellule ingrossate e con inclusi riscontrate nei polmoni, reni e capillari di un neonato. Sei casi descritti nei precedenti 20 anni tutti osservati in feti/neonati con o senza concomitante sifilide. Gli AA mettono in discussione la natura protozoaria (*Entamoeba mortinatalium*) delle cellule giganti ipotizzata in precedenza. Viene anche menzionata una certa somiglianza con quanto descritto nel 1904 in lesioni da varicella ma l'ipotesi infettiva non trova sostegno: «*The possibility of an unknown intracellular infection stimulating the cell to assume this unusual structural variation has at present no evidence to support it*.» (2)
- **1956** Vengono isolati i primi ceppi virali da bambini con malattia da inclusioni citomegaliche come pure da bambini asintomatici (3-5)
- **1960** Viene proposto il nome di citomegalovirus
- **1974** Viene pubblicato il lavoro «Development of a vaccine against mental retardation caused by cytomegalovirus infection *in utero».* (6)
- **1981** Inizio epidemia AIDS**:** HCMV diventa una causa maggiore di morte
- **1985** Sviluppo di tecniche rapide di isolamento/identificazione del virus (7)
- **1988** Prime applicazioni della tecnica PCR per la ricerca di DNA nelle urine dei neonati (8)
- **2022** Numerosi vaccini sperimentali, nessuno disponibile**.**

La prognosi dei pazienti immunocompromessi è molto migliorata grazie a farmaci HCMV-specifici e ai protocolli di monitoraggio virologico e immunologico

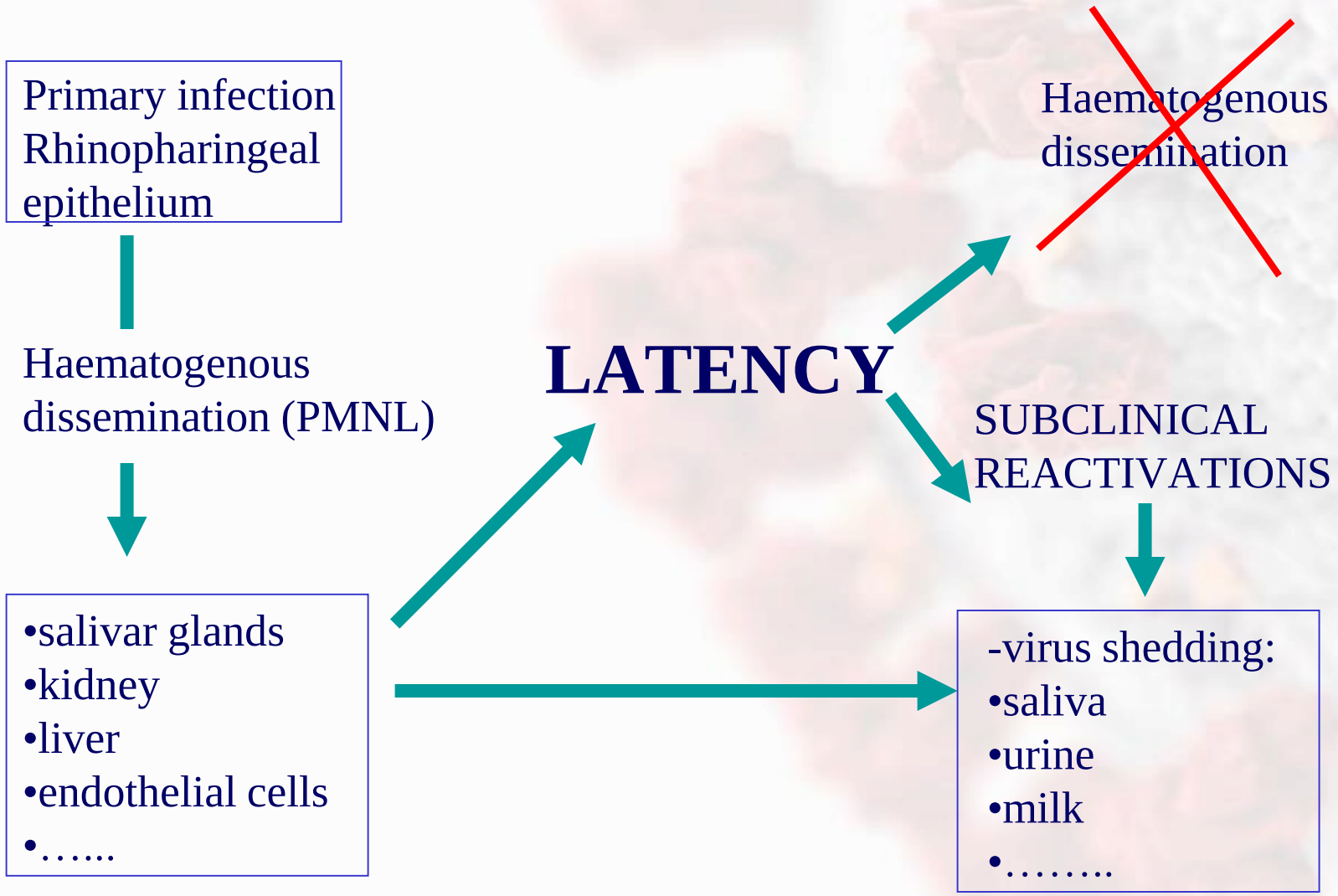
HCMV resta la prima causa infettiva di sordità e ritardo mentale congenito

2. Goodpasture EW and Talbot FB. Am. J. Dis. Child. 21:415-21, 19215. Weller TH et al., Proc. Soc. Exp. Biol. Med. 94:4-12, 1957
8. Demmler G., J. Infect. Dis. 158:1177-84, 1988
3. Rowe WP et al., Proc. Soc. Exp. Biol. Med. 92:418-422, 1956.
6. Elek SD and Stern H, Lancet 303:1-5, 1974
4. Smith MG, Proc. Soc. Exp. Biol. Med. 92:424-430, 1956
7. Gleaves CA et al., J. Clin. Microbiol. 21:217-21, 1985

Principali caratteristiche epidemiologiche

- SPECIE-SPECIFICO
- UBIQUITARIO
- SIEROPREVALENZA AUMENTA CON L'ETA' (≈50-60% donne italiane in età fertile è positiva)
- BASSA PATOGENICITA' (nel soggetto immuncompetente)
- PREVALENTE TRASMISSIONE PER VIA ORALE E SESSUALE
- TRASMISSIONE ORIZZONTALE E VERTICALE
- PROLUNGATA ESCREZIONE DEL VIRUS NEI SOGGETTI INFETTI
- MASSIMA CIRCOLAZIONE NEI BAMBINI (implicazioni per infezione materna)

HCMV infection-immunocompetent subjects

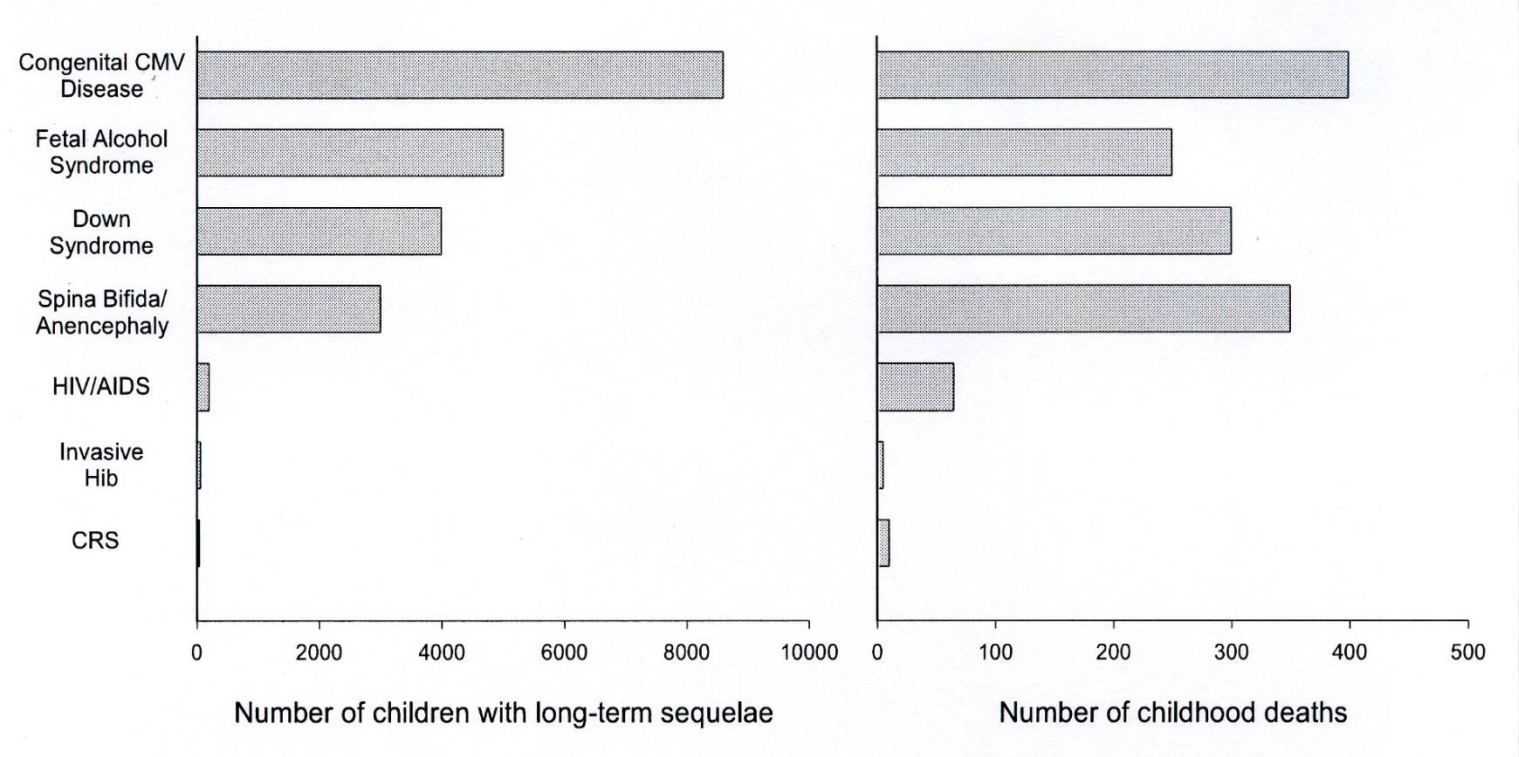


Sintomi (immunocompetente)

- febbre
- cefalea
- astenia
- mialgia
- faringite
- linfoadenopatie
- epato-splenomegalia

- ↑transaminasi
- linfocitosi

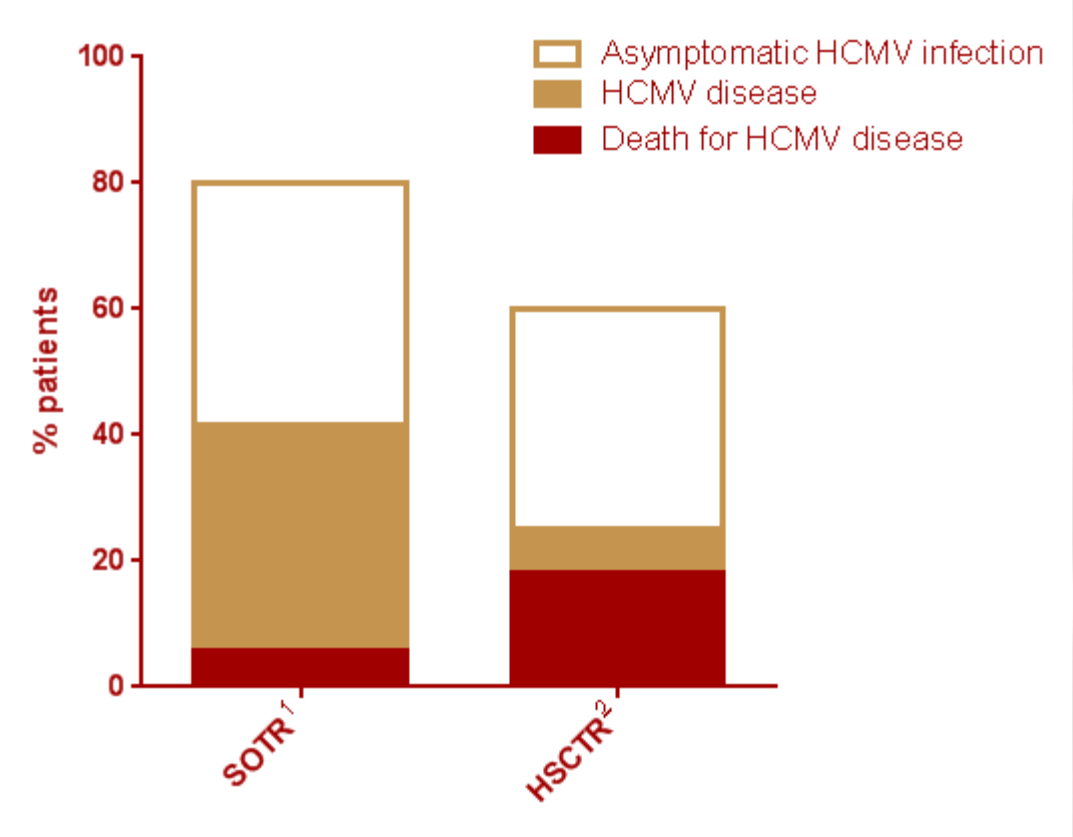
Estimates of the annual burden of prominent childhood diseases and syndromes in the US



Cannon MJ, Davis KF. BMC Public Health 2005;5:70.

The development of a CMV vaccine was listed as a top priority by the Institute of Medicine of the National Academy of Sciences (USA).
The National Vaccine Advisory Committee emphasize the financial and humanitarian justifications for developing such a vaccine.
(Stratton K et al. Washington DC, National Academy Press, 2001)

Incidence, morbidity and mortality of HCMV infection



- HCMV syndrome
- Pneumonia
- Gastro-intestinal disease
- Hepatitis
- Retinitis
- Disseminated infection

[1]*Rubin et al., Transplantation 1977; 24:458-64; Rubin RH. Rev Infect Dis. 1990;12:S754-66. Hibberd PL, et al., Ann Intern Med 1995;123:18-26; Grossi et al., Transplantation 1995; 59847-51.*

[2]*Meyers et al., JID 1986; 153:478-88; Meyers et al., NEJM 1991; 325:1601-7; Einsele et al., Blood 1995; 86:2815-20.*

Diagnosis of HCMV infection during pregnancy (immunocompetent subjects)

Diagnosis of primary HCMV infection

Presence of 2 or more of the following:

- **Seroconversion** (appearance of HCMV-specific IgG in a seronegative subjects*)
- **HCMV-SPECIFIC IgM****
- **HCMV-SPECIFIC IgG with low avidity**
- **HCMV DNA in blood*****

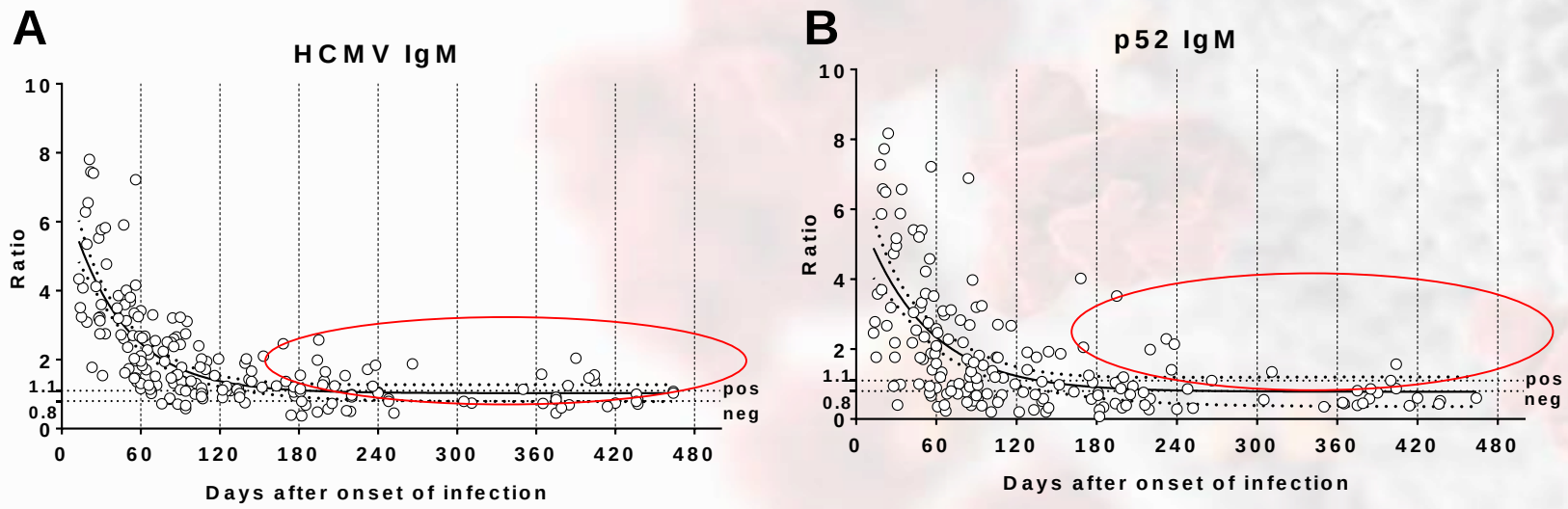
Sequential samples should be examined for a correct diagnosis (antibody kinetics)

*Pay attention to the detection of isolated or low level IgG.
**Detection of IgM may indicate: i) recent primary infection; ii) previous primary infection (IgM may persist for months in some individuals); policlonal activation or cross-reactivity during other infections (EBV, parvovirus B19).
***HCMV DNA in other bodily fluids has no relevance, since it can be detected for a long time after infection and during non-primary infections. HCMV in blood is usually present only aftr primary infction in immunocompetent subjects.

When does a primary HCMV infection ends?

Zelini et al., J Clin Virol. 2019 Nov;120:38-43.

Primary infection

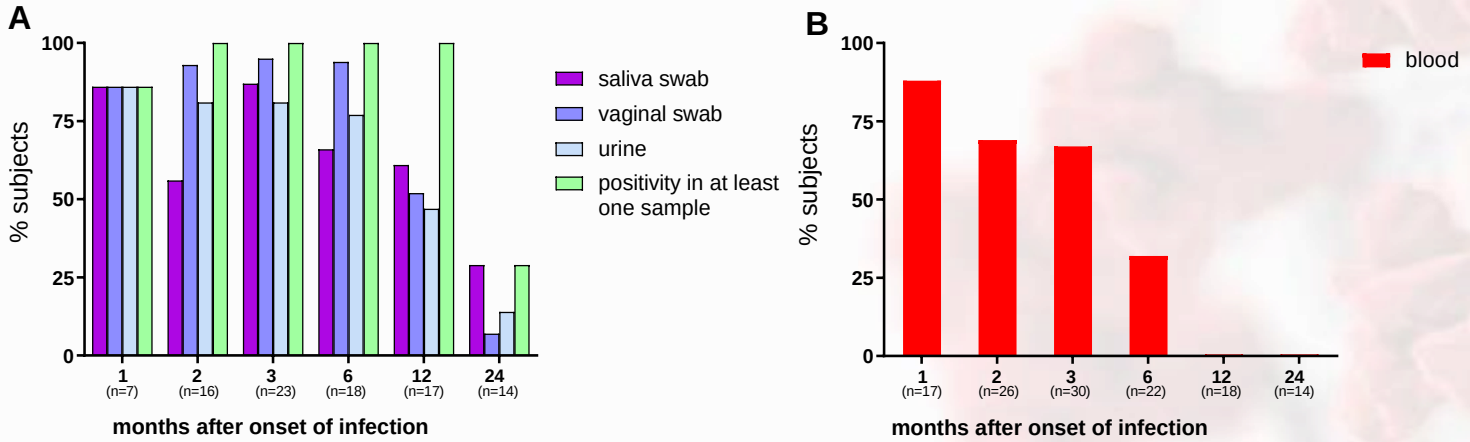


HCMV IgM were still positive in 20/45 (44%) samples collected after 180 days and in 6/16 samples (38%) collected after 360 days.
p52-IgM were still positive in 9/45 (20%) samples collected after 180 days and 3/16 samples (19%) collected after 360 days.

HCMV replication and shedding after primary infection

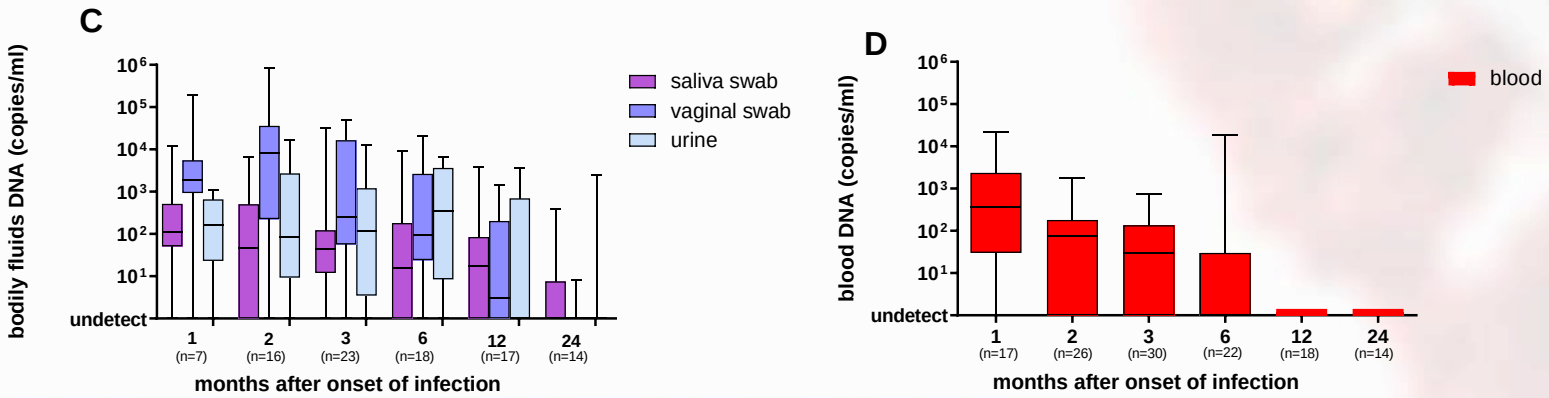
Fornara et al., Med Microbiol Immunol. 2022;211:249-260

Frequency of HCMV shedding in bodily fluids and blood



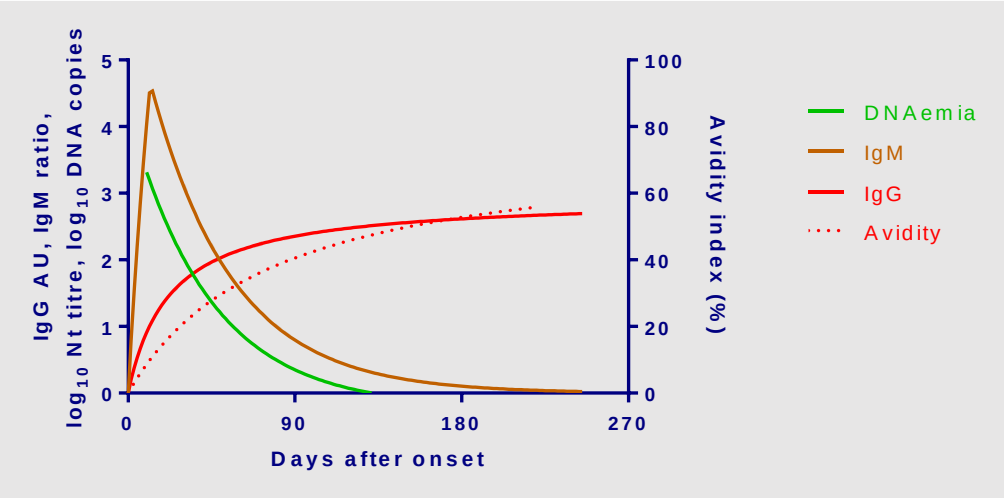
All the women with primary infection tested were still shedding HCMV DNA 12 months after onset of infection in at least one bodily fluid

Viral load in bodily fluids and blood



Dating HCMV infection onset during pregnancy

➢ Antibody and HCMV DNA kinetics during primary infection



IgM. Persists for 3-6 months. Caveat: individual variability with very fast (<1month) or slow (>12 months) responses.
DNAemia. Persists for 1-3 mesi. Caveat: Low levels and intermittent positivity
IgG avidity. Progressive maturation from low to high in 3-6 months. Low avidity: infction within the previous 3 months. Caveat: individual variability with very fast (high avidity in 1-2 months) or slow (low avidity >6 months) maturation.

Parameters and results	**presumed onset***
- IgM +, IgG -/low (avidity non-determinable), DNAemia	<4 weeks
- IgM +, IgG + (avidity low), DNAemia+	<12 weeks
- IgM +, IgG+ (avidity high) DNAemia -	unknown (likely >12 weeks)**

* with respet to the sample examined; ** IgM to be interpreted

Dating HCMV infection onset during pregnancy

- **Symptoms and signs (permit to define the onset of infection in the context of serological and virological data)**

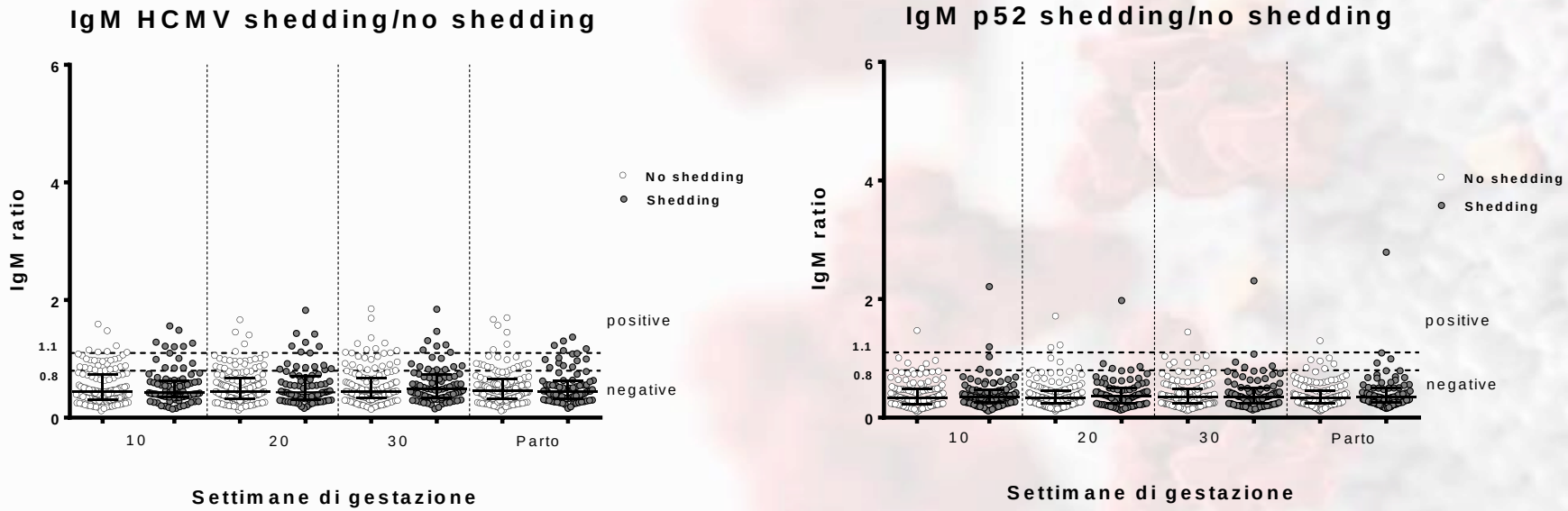
Clinical and laboratory findings in 244 pregnant women with primary HCMV infection

Symptoms or abnormal laboratory findings	no. (%) of women
Absent	78 (32.0)
Present	166 (68.0)
Fever	100 (60.2)
Fatigue	81 (48.8)
Headache	44 (26.6)
Arthralgiae/Myalgiae	25 (15.1)
Rhinitis	25 (15.1)
Pharyngitis	23 (13.9)
Cough	16 (9.6)
Elevated liver enzymes	60 (36.1)
Lymphocytosis	20 (12.0)
Cumulative no. of symptoms/abnormalities per subject	
One	49 (29.5)
Two	48 (28.9)
Three or more	70 (42.2)

Fever, headache and fatigue are the symptoms more frequently remembered.

Revello MG and Gerna G. Clin. Microbiol. Rev. 15:680-715, 2002

HCMV-IgM or IgG were not associated with non-primary infections.



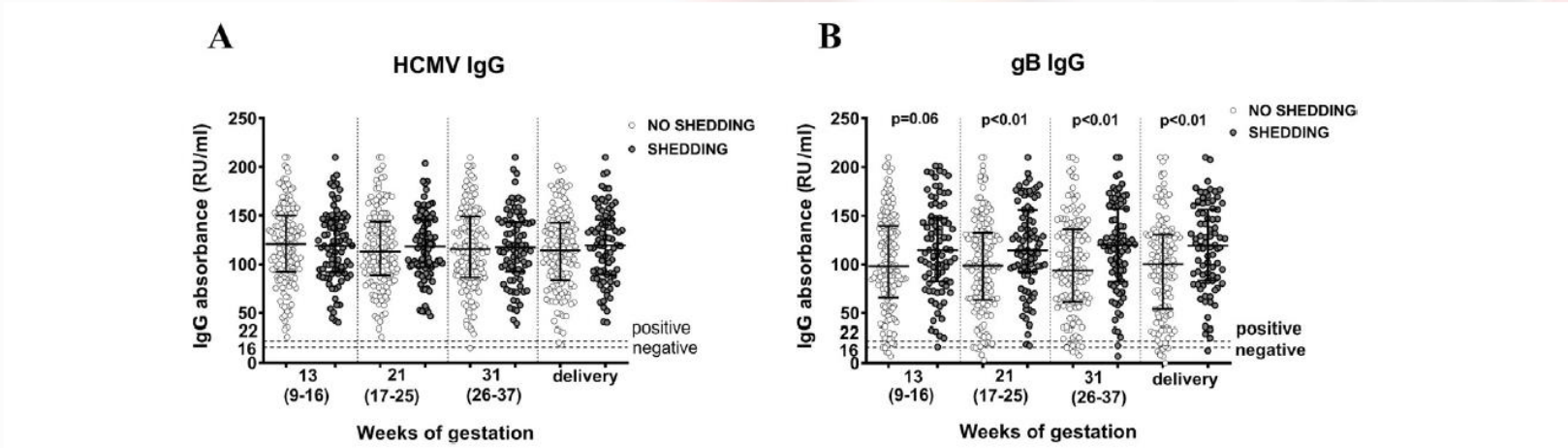


Fig. 4. Levels of IgG (A) and IgG anti-gB (B) by ELISA in seropositive pregnant women with or without non-primary HCMV infection (NPI) at all times. Kruskall–Wallis test and Dunn's *post hoc* test with correction for multiple comparisons were used for statistical analysis. Only p values ≤ 0.10 were reported.

Diagnosis of congenital HCMV infection

Antenatal

HCMV DNA in amniotic fluid

At birth

HCMV DNA in urine within 3 weeks of age

HCMV DNA in saliva as screening to be confirmed in urine (< 3 weeks of age) in case of positive result

Retrospective

HCMV DNA in dry blood spots collected at birth

Prevention of congenital HCMV infection

Several vaccines in development. None available.

Prevention of maternal infection.

Hygienic behaviour intervention. Derived from the knowledge of the major route of infection (contact with child saliva and urine).

May reduce by 90% the risk for primary infection

Prevention of fetal infection.

- Valaciclovir

  (2020) Israel. Randomized study: 8 g/day. Reduction of transmission from 30% to 11% (p=0.027) at amniocentesis.

- Hyperimmun globulin

  (2019) Germany. Observational study: 200 U/Kg i.v. every 15 days. Reduction of HCMV transmission from 35.2% a 7.5% ($p<0.0001$).

Serological screening for HCMV infection

- pre-conceptional in women of child-bearing age (best option);
- at delivery for women with unknown serostatus;
- at the beginning of pregnancy, immediately after pregnancy test for pregnant women with unknown serostatus.

Diagnosis of HCMV infection in transplant recipients (immunocompromised subjects)

Rilevazione di HCMV nel sangue

-Soggetti immunocompetenti:

quasi esclusivamente durante un'infezione primaria

-Pazienti immunocompromessi:

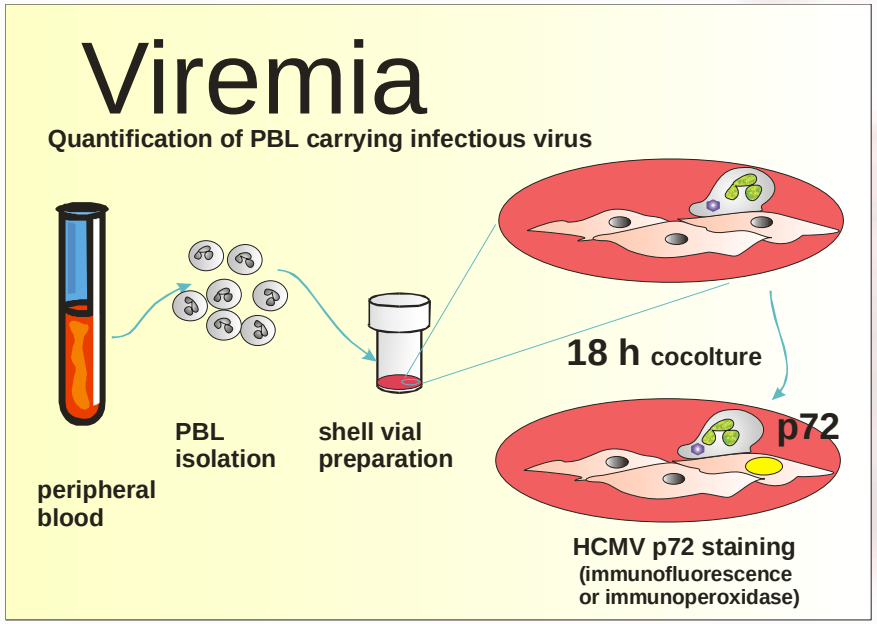
Sia durante in infezione primaria sia
durante la **maggior parte** degli episodi di riattivazione

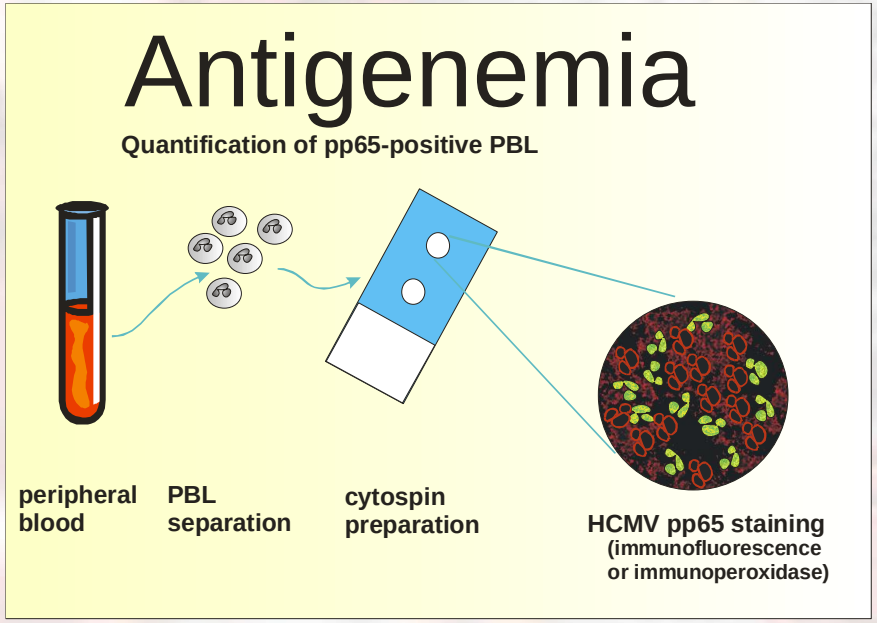
Il monitoraggio della presenza di HCMV nel sangue permette di identificare i pazienti a rischio di sviluppare una malattia da HCMV. Lo sviluppo di sintomi correla con la carica virale nel sangue

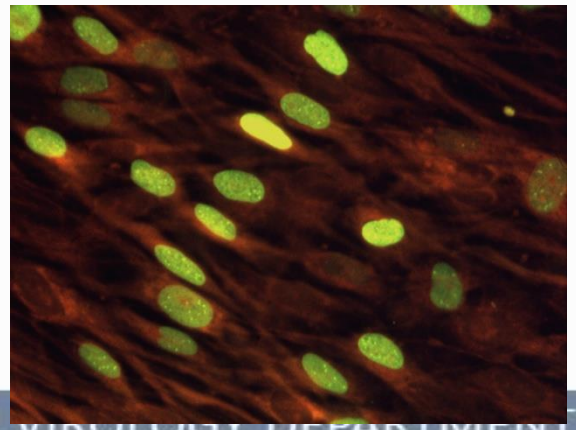
Breakthrough in early '90s: rapid diagnosis of HCMV infection

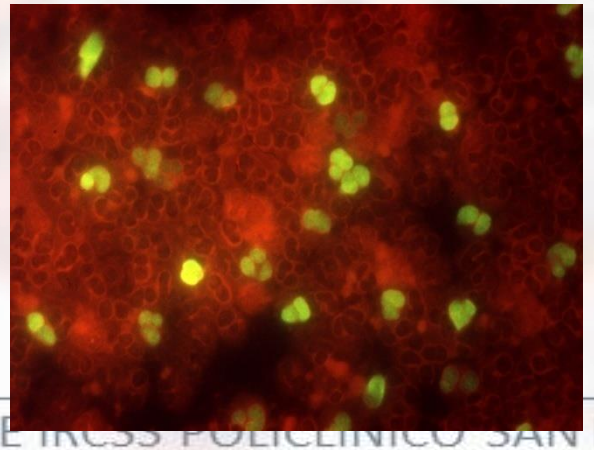
Gerna et al., J Clin Microbiol, 1990; 28: 2681-2688.

van der Bij et al., Transplant Proc. 1989;21:2061-4
Gerna et al., J Clin Microbiol, 1992; 30: 1232-1237.









Saggi per la ricerca di HCMV nel sangue

- **Viremia**
  (rilevazione di virus infettante)
- **Antigenemia**
  (rilevazione dell'antigene pp65 nei leucociti)
- **DNAemia**
  (rilevazione del DNA di HCMV)

La ricerca del virus in altri fluidi biologici (saliva, urine) non ha rilevanza clinica. La sierologia non è utile per la diagnosi di infezione attiva da HCMV nel paziente trapiantato. Serve solo per stabilire il rischio di infezione/malattia in base al sierostato donatore /ricevente pre-trapianto

Diagnosi di malattia da HCMV (in presenza di sintomi)

- **Sindrome sistemica**:- sintomi/segni (febbre>38°C, malessere, leucocitopenia, trombocitopenia, aumento transaminasi seriche)

  - presenza di HCMV nel sangue (DNAemia, pp65-antigenemia, isolamento virale)

  - assenza di altre possibili cause

- **Malattia d'organo**: - sintomi/segni compatibili

  - presenza di HCMV* nella biopsia tissutale (isolamento virale, istopatologia, immunoistochimica)

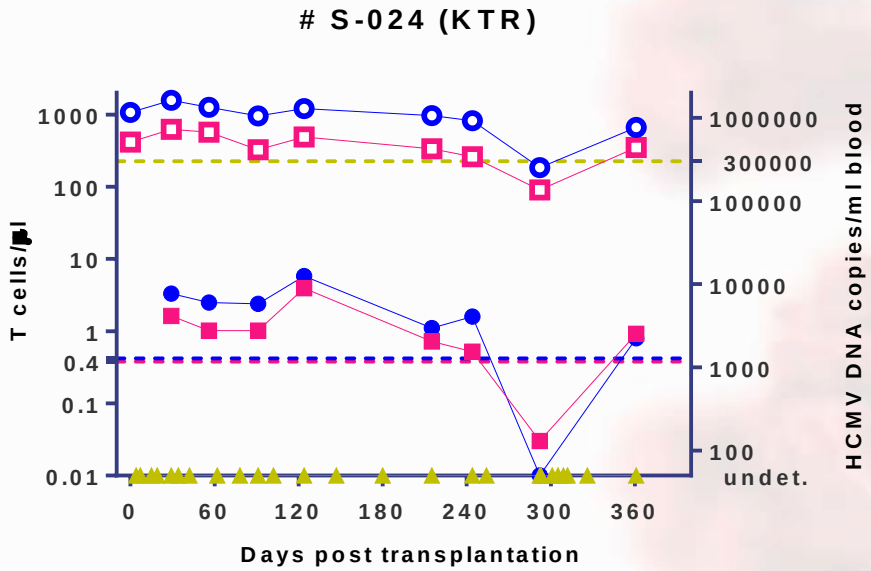
  - assenza di altre possibili cause

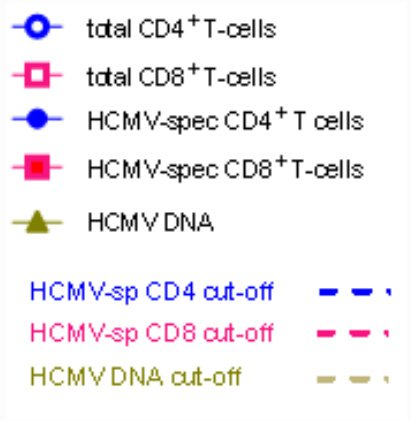
La malattia d'organo (in particolare gastroenterite) può avvenire anche in assenza di virus rilevabile nel sangue o in presenza di bassa carica virale.

* La diagnosi di malattia da HCMV è definita come: a) certa, b) probabile o c) possibile. La determinazione quantitativa del DNA di HCMV mediante realtime PCR può definire la malattia da HCMV come probabile o possibile, mentre l'isolamento, l'analisi istopatologica o immunoistochimica sono necessarie per la definizione di certezza (Ljungman et al., CID 2017;64:87–91)

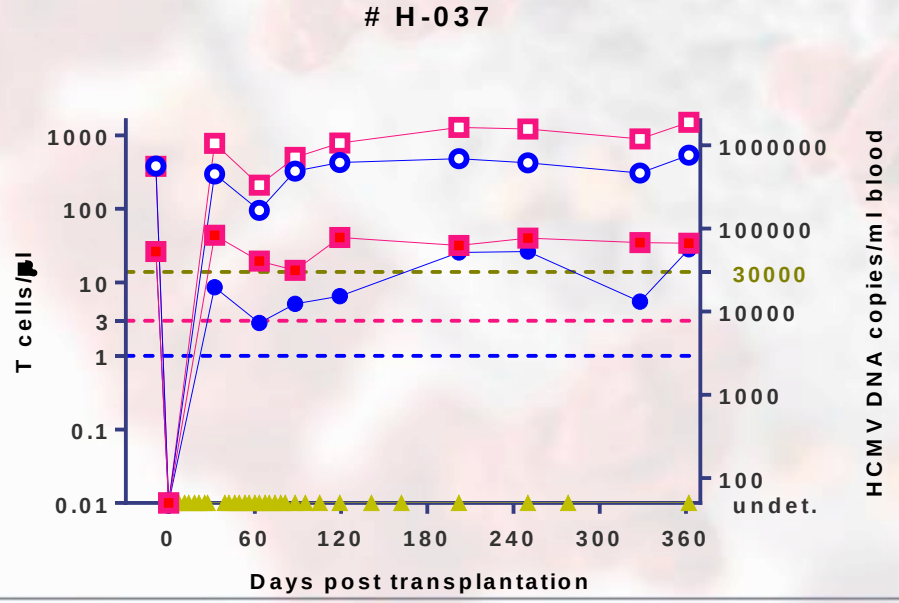
L’infezione da Citomegalovirus nel paziente trapiantato:

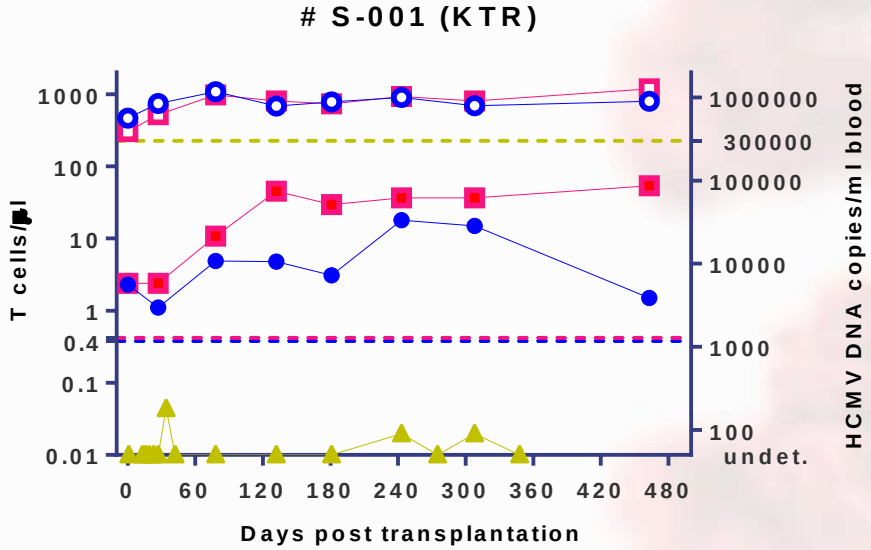
casi clinici



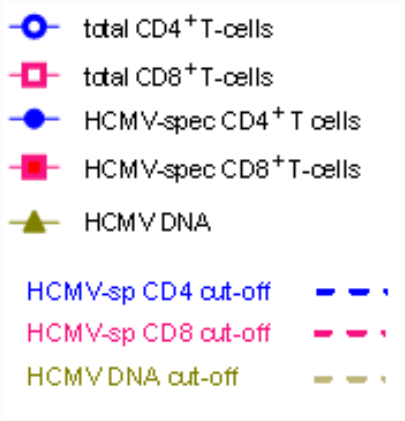


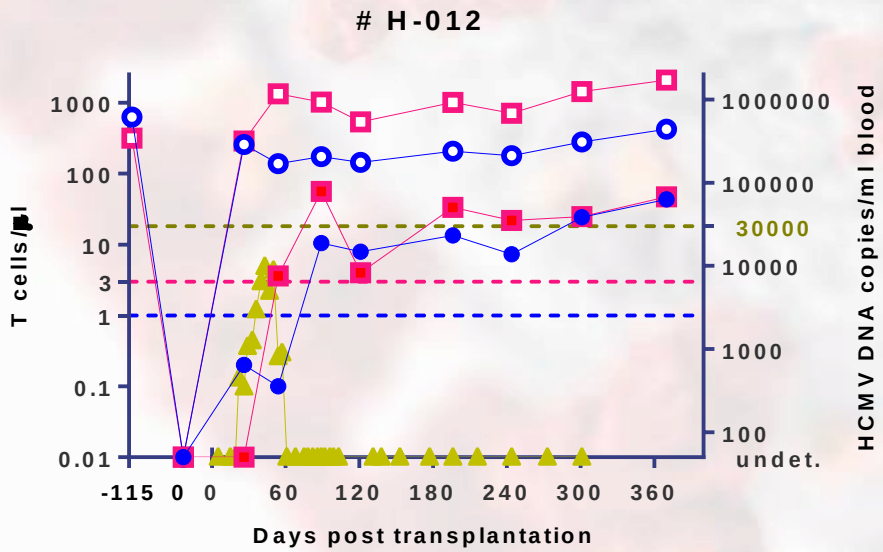
Nessuna riattivazione



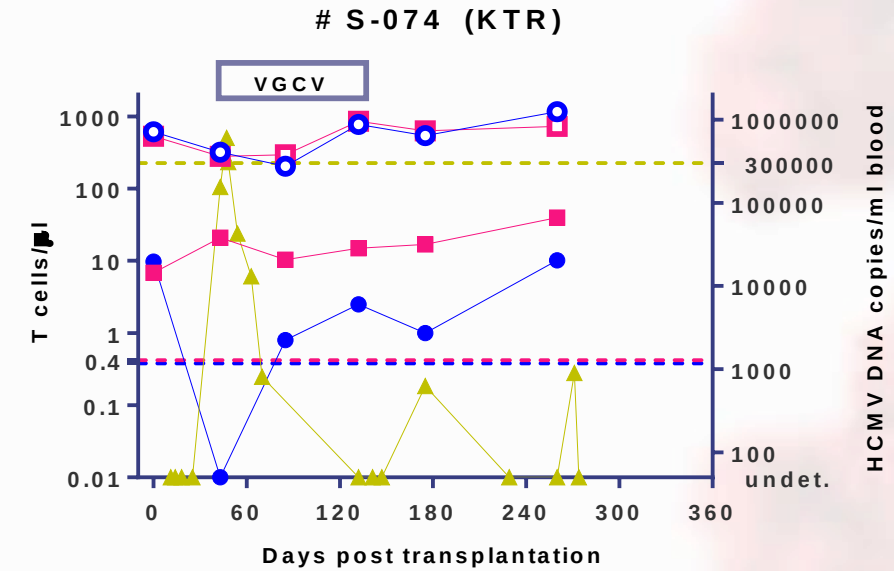


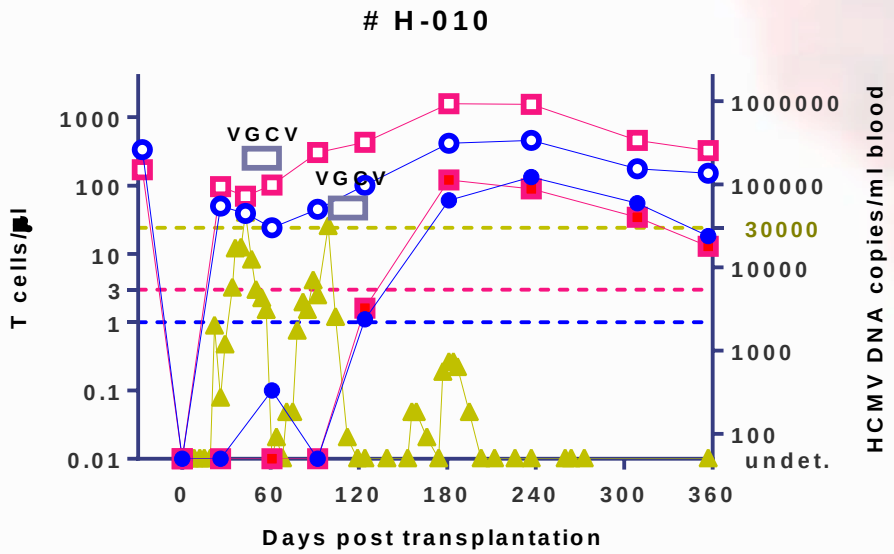
Infezione controllata

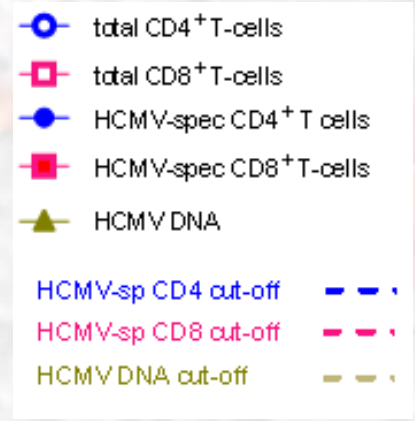




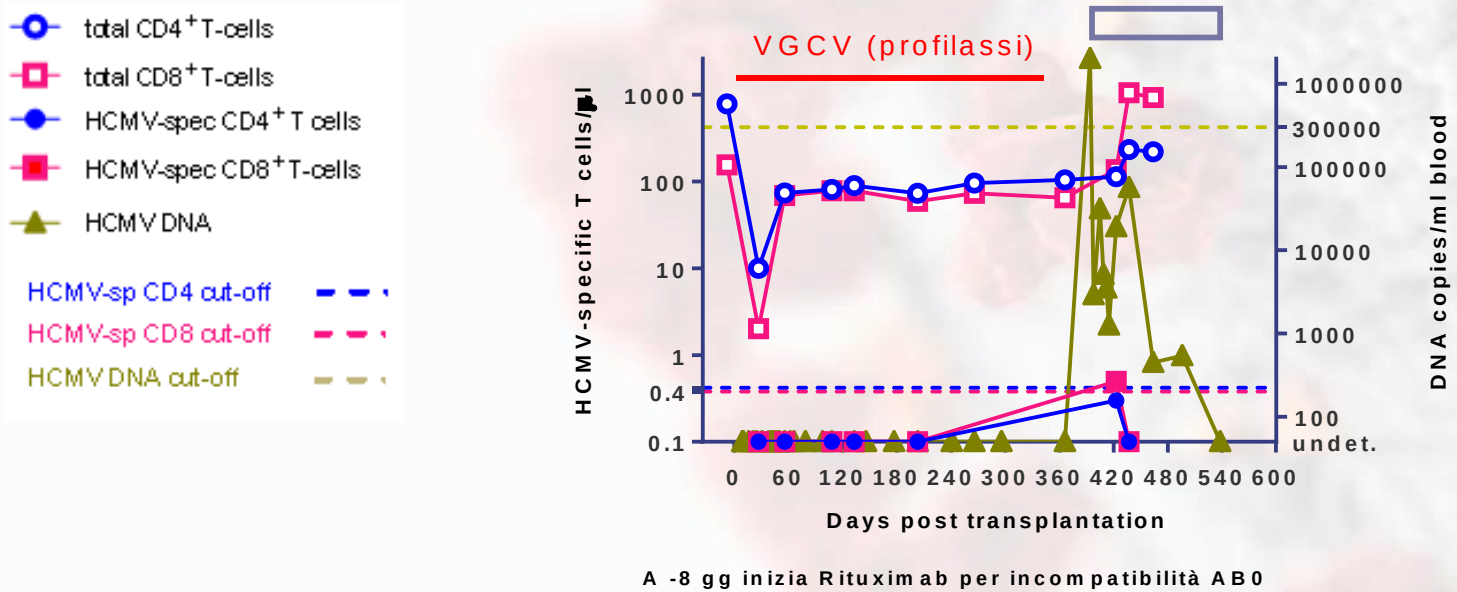
Infezione clinicamente significativa trattata con antivirale







KTR D+/R+ dopo profilassi con VGCV



Virus Research 104 (2004) 129–137

Detection of RNA in purified cytomegalovirus virions

Elizabeth Sarcinella[a,b], Martha Brown[c,1], Raymond Tellier[a,d,2], Martin Petric[e,3], Tony Mazzulli[a,b,*]

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[b] TML/MSH Department of Microbiology, Mount Sinai Hospital, 600 University Ave., Room 1485, Toronto, Ont., Canada M5G 1X5
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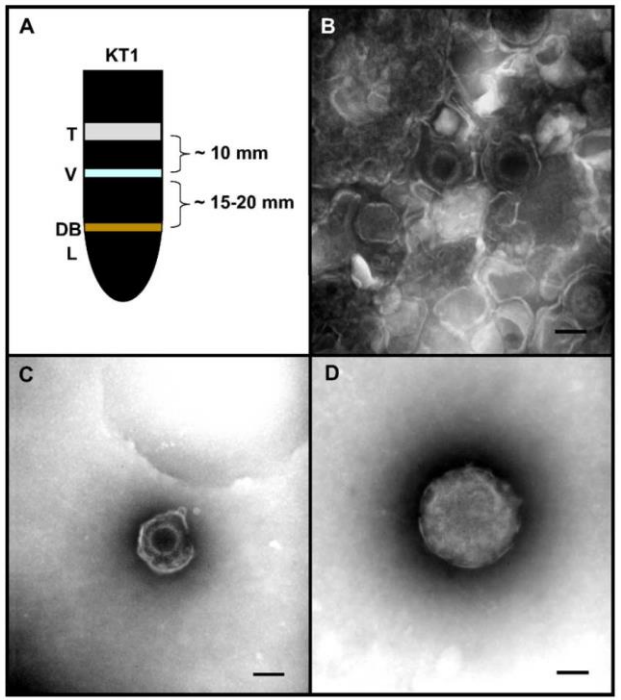
[d] Department of Pediatric Laboratory Medicine, The Hospital for Sick Children, 555 University Ave., Room 7266, Toronto, Ont., Canada M5G 1X8
[e] Department of Pathology and Laboratory Medicine and British Columbia Center for Disease Control, University of British Columbia, 655 W 12th Ave., Vancouver, BC, Canada V5Z 4R4



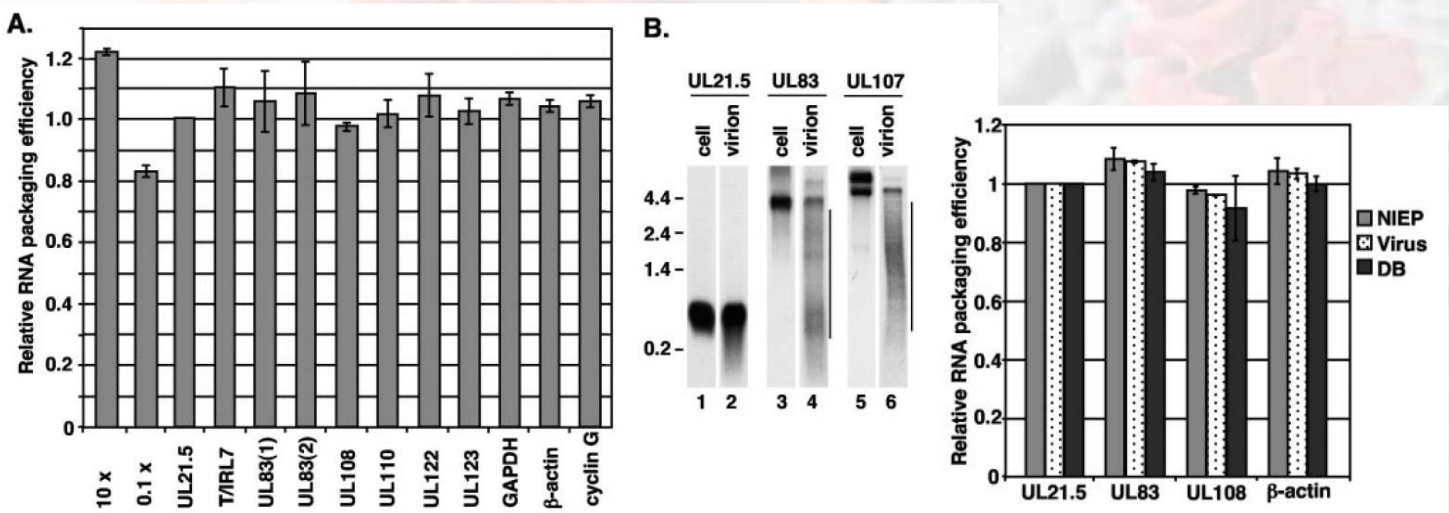
(A) The banding pattern observed following ultracentrifugation. (B) The top band in the gradient (T), contained virions/NIEPs trapped within cellular debris. (C) The middle band (V), consisted of virions/NIEPs (and small DBs, not shown) in the absence of debris. (D) The lower band (DB), consisted of approximately 95% DBs, in the absence of debris. Bars, 100 nm.

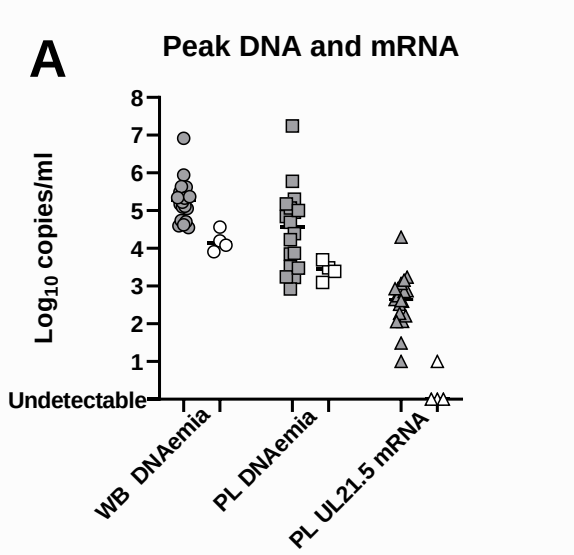
JOURNAL OF VIROLOGY, Oct. 2004, p. 10390–10398 Vol. 78, No. 19
0022-538X/04/$08.00+0 DOI: 10.1128/JVI.78.19.10390–10398.2004

RNAs Are Packaged into Human Cytomegalovirus Virions in Proportion to Their Intracellular Concentration

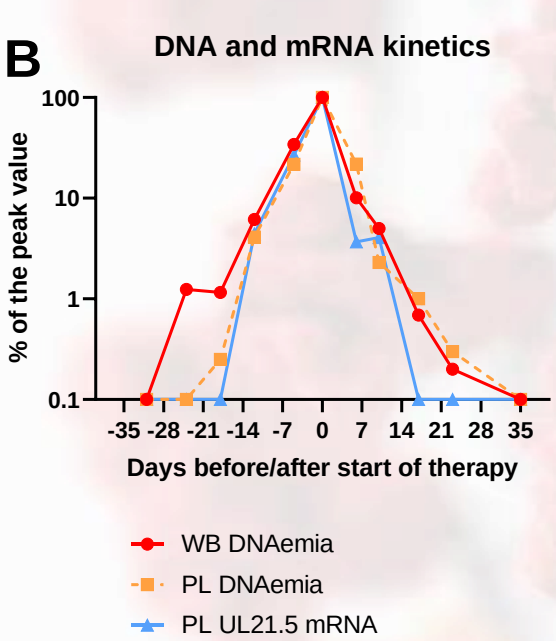
Scott S. Terhune, Jörg Schröer, and Thomas Shenk*

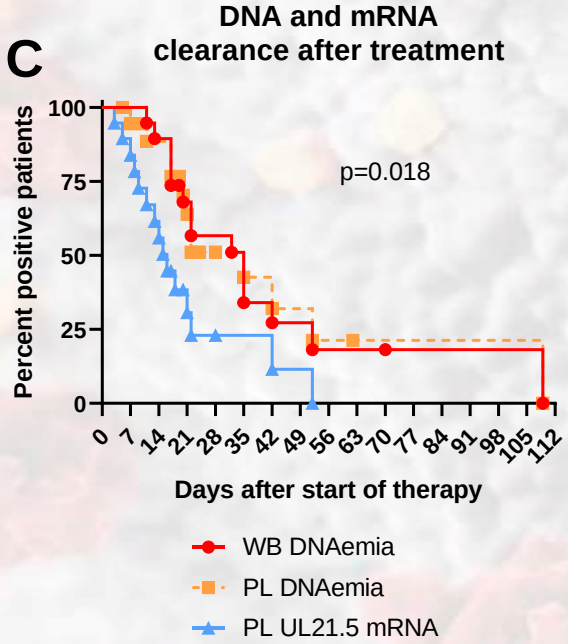
Department of Molecular Biology, Princeton University, Princeton, New Jersey





Grey symbols: clinically significant DNAemia
White symbols: transient self-resolving DNAemia



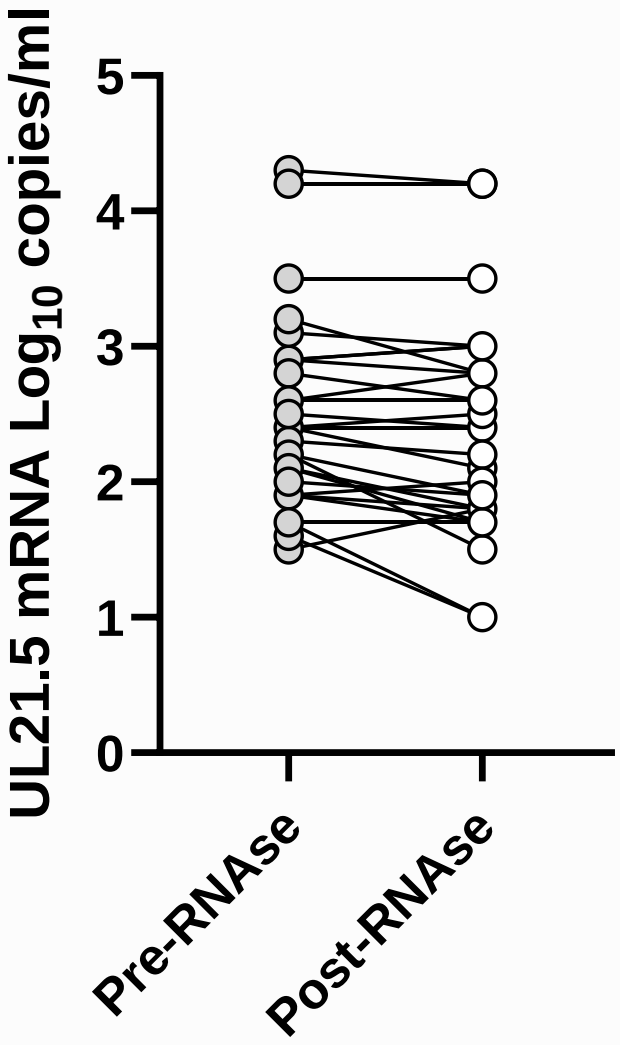


UL21.5 mRNA was detected in plasma 1-2 weeks before administration of pre-emptive therapy in all the 20 episodes of clinically significant DNAemia.

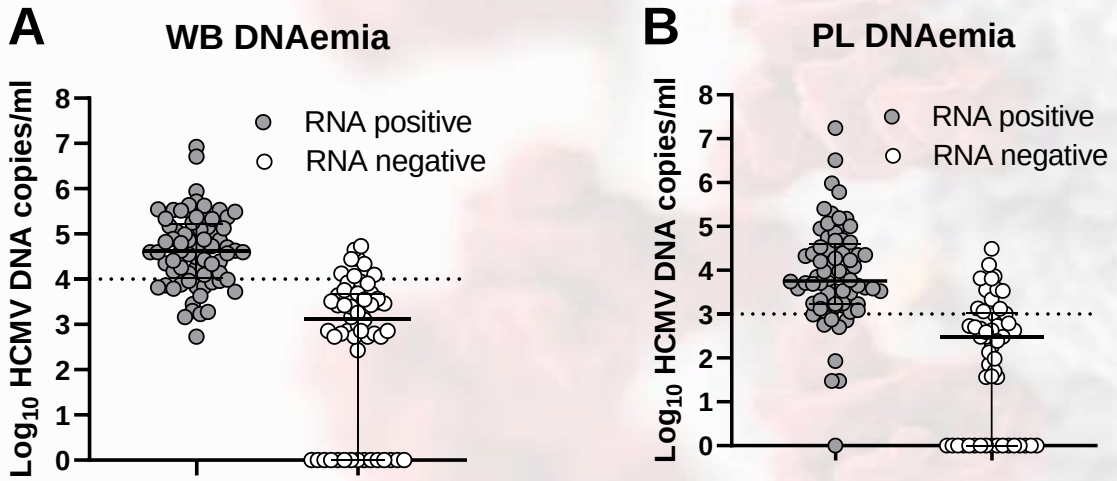
UL21.5 mRNA was detected about two weeks after WB DNAemia appearance, and, during pre-emptive therapy, it was cleared earlier than WB DNAmia.

After start of treatment, median time to UL21.5 mRNA clearance was 16 days, whereas median time to WB DNAemia and PL DNAemia was 36 days.

RNAse protection assay



We tested UL21.5 mRNA in plasma before or after RNAse digestion to verify whether it is free-floating in plasma (and therefore undetectable after digestion) or packaged in virions (and therefore resistant to RNAse digestion).
After RNAse digestion of the plasma, UL21.5 mRNA was still detectable in all samples, with a median reduction of 0.1 (IQ range 0-0.3) Log10. This indicates that the UL21.5 mRNA detected in plasma is mainly packaged in virions



Considering cut-off values of 10^4 copies/ml for WB DNAemia or 10^3 copies/ml for PL DNAemia, 75% of samples positive for UL21.5 mRNA had WB and PL DNAemia values above these cut-off values. Conversely, only a few samples that were negative for UL21.5 mRNA had DNAemia values above 10^4 or 10^3 copies/ml WB or PL (10% and 25% of samples, respectively).