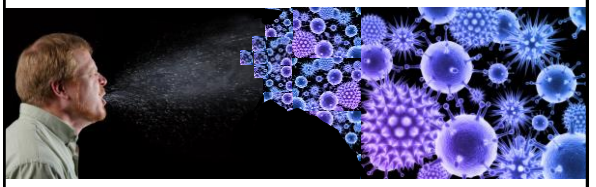
Influenza and exanthematic viruses



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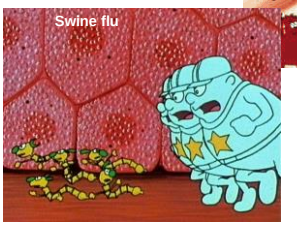
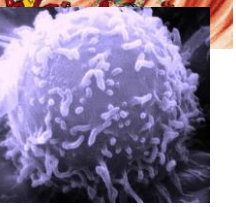
Life is fight





Viruses described in immunosupressed patient

RNA viruses

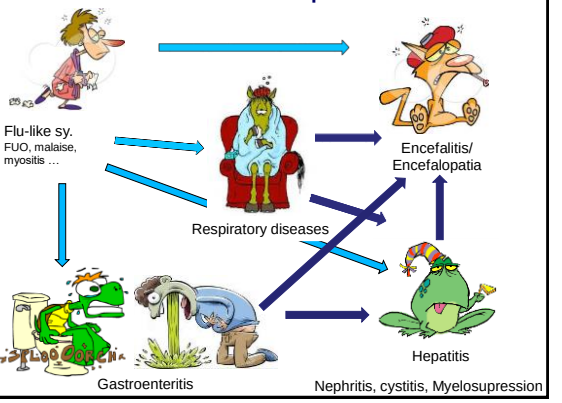
ss RNA

- *Ortomyxoviridae* → Influenza A, Influenza B
- *Paramyxoviridae* → Paramyxovirus → PIV; Morbillivirus; Pneumovirus → RSV, hMPV
- *Coronaviridae* → HCoV
- *Picornaviridae* → Enteroviruses; Rhinovirus → HRV
- *Caliciviridae* → Human caliciviruses
- *Astroviridae* → Astrovirus
- *Rhadboviridae* → Lyssa virus
- *Flaviviridae* → HCV…

ds RNA

- *Reoviridae* → Rotavirus, Orbivirus

Clinical consequences



What to aim during the process of dg? Clinical symptoms

Adapted ECDC Definitions of Respiratory Tract Infectious Disease (RTID)

Clinical criteria

- *New* onset of symptoms
  AND
  at least one of the following four respiratory symptoms:
  - Cough
  - Sore throat
  - Shortness of breath
  - Coryza
  AND
  - A clinician's judgement that the illness is due to an infection

Epidemiological Criteria

- An epidemiological link with human to human transmission

Laboratory Criteria

- Detection of CARV in a clinical specimen by at least *one* of the following:
  - Virus isolation by cell culture (VIC)
  - Direct virus antigen testing (DAT)
  - Nucleic acid amplification testing (NAT)

Case Classification

- **Possible case**
  - Any person meeting the clinical criteria of RTID
- **Probable case**
  - Any person meeting the clinical criteria of RTID *and* with an epidemiological link
- **Confirmed case**
  - Any person meeting the clinical of RTID *and* the laboratory criteria

Adapted from ECDC definitions for influenza
http://ecdc.europa.eu/en/activities/surveillance/EISN/surveillance/Pages/influenza_case_definitions.aspx

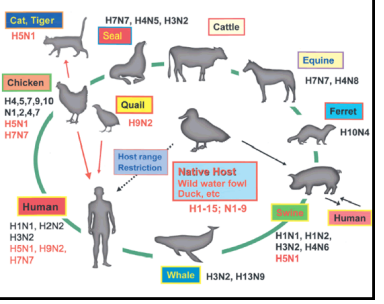
4th European Conference on Infections in Leukemia

Respiratory viruses

- Often zoonotic:
  - SARS – CoV
  - MERS - CoV
  - …

Bats – Horseshoe bat,…

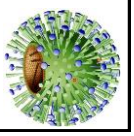
Civet



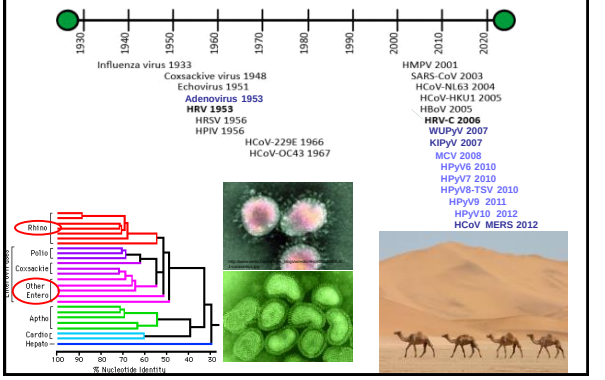
Leads to high Frequence of recombination of new life-threatening infections

What is influenza?

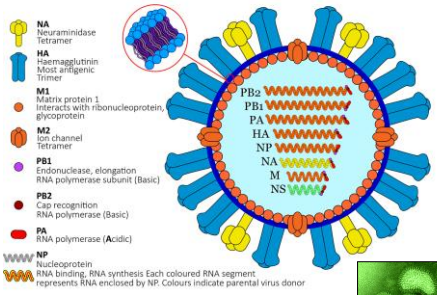
- An acute respiratory illness resulting from infection with an influenza virus (Orthomyxoviruses)
- Highly infectious and can spread rapidly from person to person
- Some strains cause more severe illness than others
- Highly infectious viral illness
- 412 BC - first mentioned by Hippocrates
- 1580 - first pandemic described
- 1580-1900 - 28 pandemics
- Virus first isolated in 1933



History of viral respiratory infections

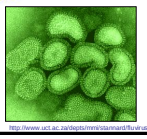


ORTHOMYXOVIRUSES



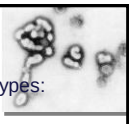
typ A, B, C : NP, M1 protein
sub-typy: HA nebo NA protein

https://figshare.com/articles/Influenza_virus/6817112



http://www.uct.ac.za/depts/mrc/starnard/flu/virus.html

Types of influenza viruses

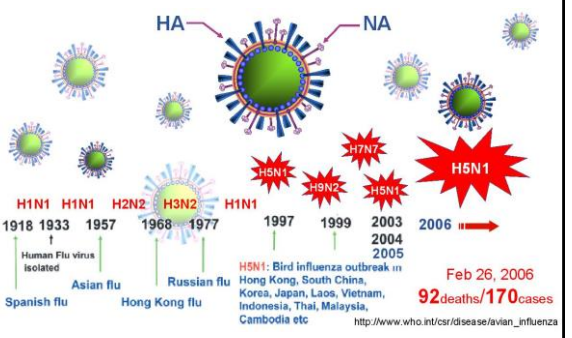


- Influenza viruses are divided into three main types: influenza A, B, and C
- **Group A** viruses
  - infect birds and other animals, as well as humans
  - source of seasonal influenza epidemics and all pandemics
  - moderate to severe illness
  - all age groups
  - humans and other animals
  - typed by NA and HA
- **Group B**
  - changes less rapidly than type A – no Ag shift
  - infects humans only, milder epidemics
  - primarily affects children
- **Group C** viruses
  - infect humans only and do not cause pandemics

Types of influenza viruses

	TYPE A	TYPE B	TYPE C
severity of illness	++++	++	+
animal reservoir	yes	no	no
human pandemics	yes	no	no
human epidemics	yes	yes	no (sporadic)
antigenic changes	shift, drift	drift	drift
segmented genome	yes	yes	yes
amantadine, rimantidine	sensitive	no effect	no effect
zanamivir	sensitive	sensitive	
surface glycoproteins	2	2	(1)

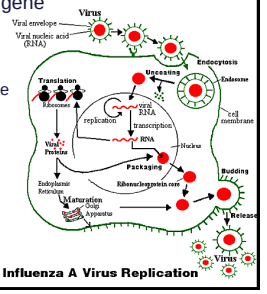
Influenza A viruses



Influenza Antigenic Changes

- **Antigenic Drift** - seasonal
  - Minor change, same subtype
  - Caused by point mutations in gene
  - May result in epidemic

Example of antigenic drift
- In 2003-2004, A/Fujian/411/2002-like (H3N2) virus was dominant
- A/California/7/2004 (H3N2) began to circulate and became the dominant virus in 2005

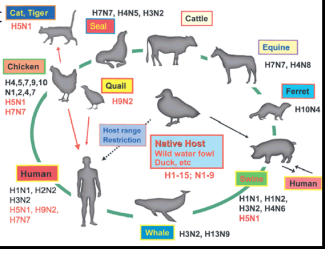


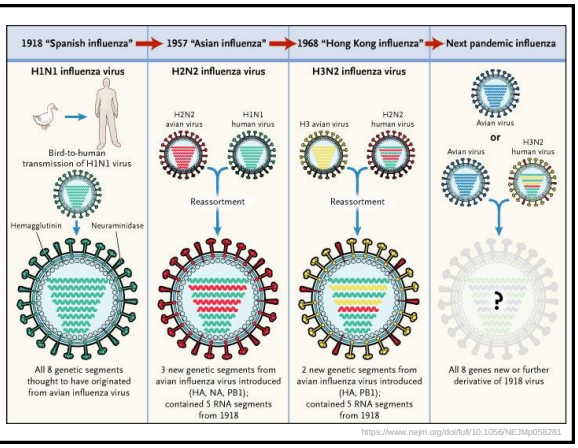
Influenza A Virus Replication

Influenza Antigenic Changes

- **Antigenic Shift**
  - Major change, new subtype
  - Caused by exchange of gene segments
  - May result in pandemic

- Example of antigenic shift
  - H2N2 virus circulated in 1957-1967
  - H3N2 virus appeared in 1968 and completely replaced H2N2 virus

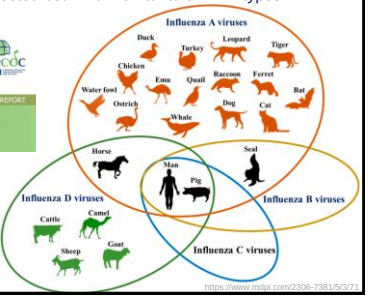




https://www.nejm.org/doi/full/10.1056/NEJMp058281

How many HA and NA?

- 13 types HA
- 9 types NA – all circulating in birds
- Pigs – might be infected both with human and bird's types



https://www.mdpi.com/2306-7381/5/3/74

Burden of Influenza

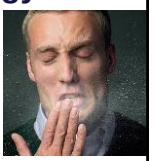
- 10% to 20% of the population is infected with influenza virus each year
- Average of more than 200,000 excess hospitalizations each year
  - Persons 65 and older and 2 years and younger at highest risk
- Average of 36,000 deaths each year
  - Persons 65 and older at highest risk of death

Influenza Associated Pulmonary and Circulatory Deaths, 1998

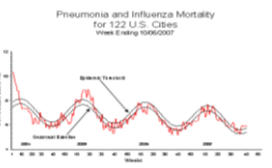
Age Group (yrs)	Rate (per 100,000)	
0 – 49	0.4 – 0.6	
50 – 64	7.5	
≥65	98.3	(>90% mortality rate)

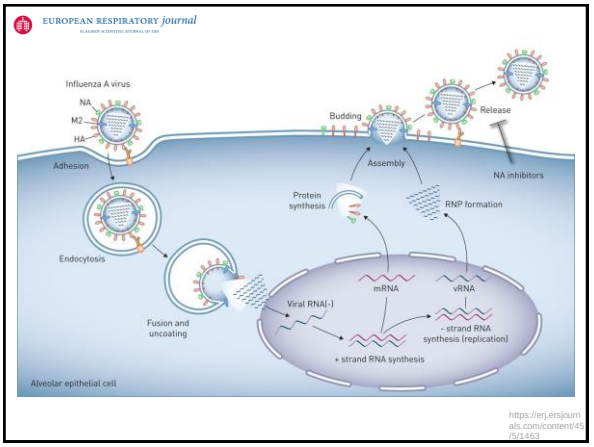
Influenza Epidemiology

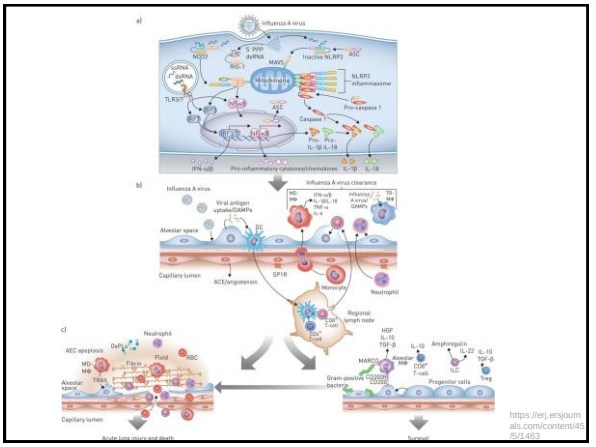
- Reservoir: Human, animals (type A only)
- Transmission: - inhaling respiratory aerosols containing the virus, produced when infected person talks, coughs, or sneezes
  100,000 - 1,000,000 virions/droplet
  » touching an infected person or an item contaminated with the virus and then touching your eyes, nose, or mouth



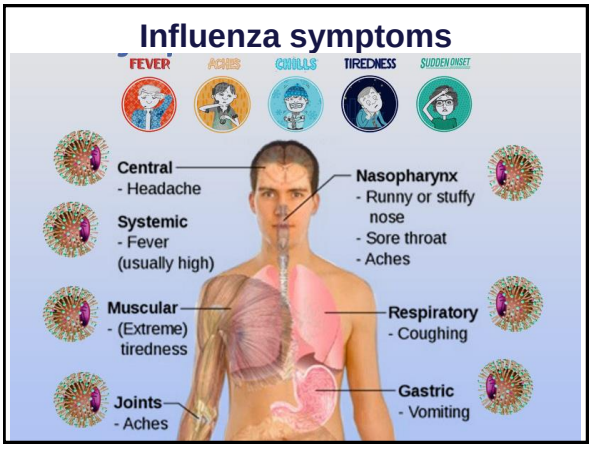
- Incubation: 18-72 hours
- Communicability: Maximum 1-2 days before to 4-5 days after onset







Influenza symptoms



Pandemic influenza in the 20th Century

1918 "Spanish Flu" 20-40 million deaths → H1N1

1957 "Asian Flu" 1 million deaths → H2N2

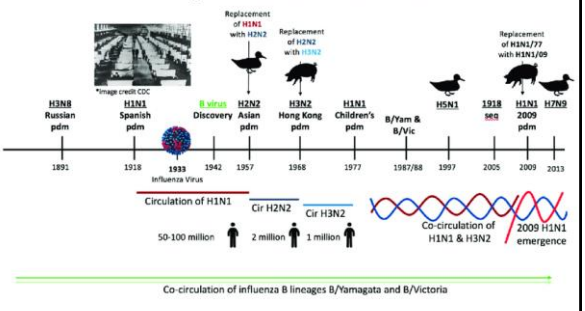
1968 "Hong Kong Flu" 1 million deaths → H3N2

1920 1940 1960 1980 2000

Influenza symptoms

- **Severity**
  - Very young (neonates) or old patients
  - Immunocompromised patient
  - Lung or heart complications

History of Influenza A and B viruses



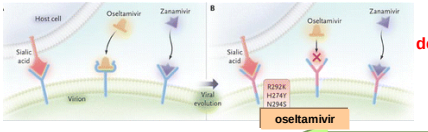
Francis, Magen & King, Morgan & Kelvin, Alyson. (2019). Back to the Future for Influenza Preimmunity—Looking Back at Influenza Virus History to Infer the Outcome of Future Infections. Viruses. 11. 122. 10.3390/v11020122.

Patient 1

Influenza A virus

Macroscopic picture of influenza pneumonia.

1st proven oseltamivir resistance in the Czech Republic.



Resistance developed after 4 weeks of therapy.

Clinical improvement

*7.1.2013 1 2 3 4 5 +12.6.2013

Complications

- **Pulmonary**
  - CROUP (YOUNG CHILDREN)
  - PRIMARY INFLUENZA VIRUS PNEUMONIA
  - ***SECONDARY BACTERIAL INFECTION***
    - *Streptococcus pneumoniae*
    - *Staphlyococcus aureus*
    - *Hemophilus influenzae*
- **Non-Pulmonary**
  - myositis (rare, > in children, > with type B)
  - ***cardiac complications***
  - recent studies report encephalopathy
    - studies of patients <21 yrs in Michigan - 8 cases seen last season
  - liver and CNS
    - Reye syndrome
  - peripheral nervous system
    - Guillian-Barré syndrome

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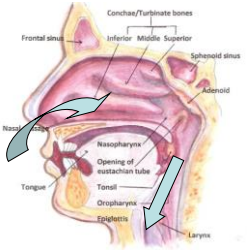
What to aim during the process of dg?

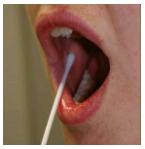
Good sampling of biological material

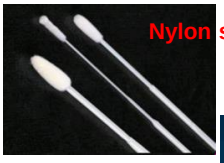
First proliferation at the mucos of upper respiratory tract.

Virus	*Transmission from upper to lower RT*	*Mortality*
RSV	20-68%	17-70%
PIV	13-37%	10-30%
HRhV	<10%	<10%

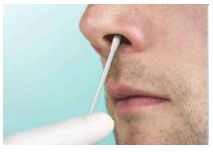


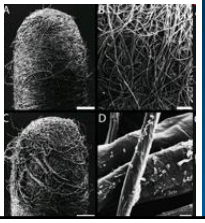
Type of swabs

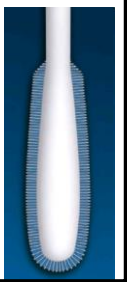




Nylon swabs

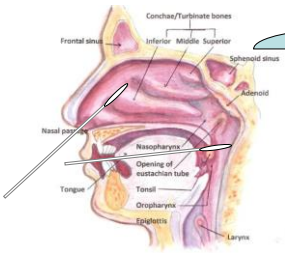


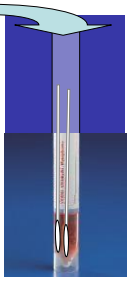




What to aim during the process of dg?

Good sampling of biological material





Diagnosis

- Virus isolation
  - Tissue culture or eggs
- Rapid tests (ussually antigen detection)
- Provisional - clinical picture + outbreak
- **PCR**

- Serology

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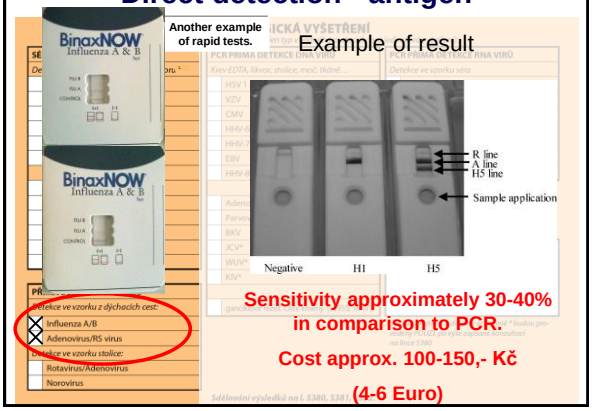
Direct detection - antigen



Sensitivity approximately 30-40% in comparison to PCR.

Cost approx. 100-150,- Kč

(4-6 Euro)

Sensitivity of antigen detection?

	Detection Ag			Detection PCR		
	No. tests	+	Discrep.	No. tests	+	Discrep.
IF-A	256	19	35	248	50	3
IF-B	256	1	3	248	4	0
RSV	207	19	47+14	248	85	1
AdV	207	3	29	248	34	2

% positive Ag vs. PCR

Influenza A = 38% RSV = 22%

Influenza B = 25% AdV = 8.8%

Using of imunochromatografi tests

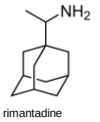
RapidVIDITEST

(RSV-Adeno, Influenza A+B)

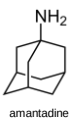
Treatment (prevention) - drugs

All virostatics have to be given early after infection

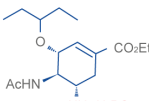
- rimantadine (M2)
  - Type A only
- amantadine (M2)
  - Type A only
- zanamivir (NA)
  - Type A and B
- oseltamivir (NA)
  - Type A and B
- peramivir (NA)
  - Type A and B



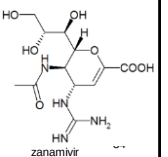
rimantadine



amantadine



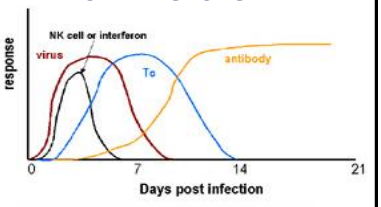
Oseltamivir Phosphate (Tamiflu)



zanamivir

Recovery

- **INTERFERON – side effects include**
  FEVER, MYALGIA, FATIGUE, MALAISE
- **CELL-MEDIATED IMMUNE RESPONSE**



Typical response to an acute virus infection

- **TISSUE REPAIR**
  can take some time

Protection against re-infection

- IgG and IgA
  - IgG less efficient but lasts longer
- antibodies to both HA and NA important
  - antibody to HA more important (can neutralize)

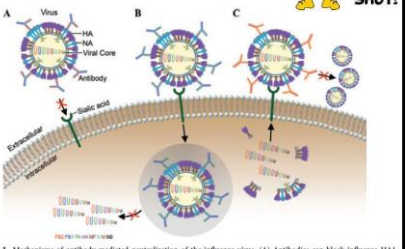
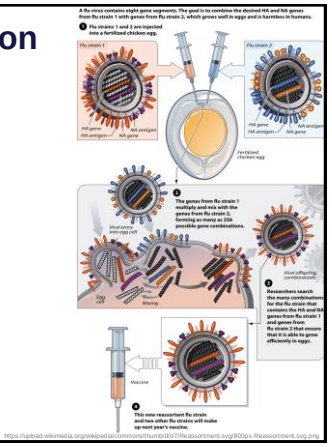


Figure 1. Mechanisms of antibody-mediated neutralization of the influenza virus. (A) Antibodies can block influenza HA1 glycoprotein binding to sialic acid residues of receptor proteins on host cells. (B) Antibodies specific to the HA2 glycoprotein of the virus can inhibit its low-pH triggered fusion activity in the endosome at the postbinding/prefusion stage, which inhibits replication of the virus. (C) Antibodies to surface neuraminidase can prevent the release of influenza virions from the infected cell surface.

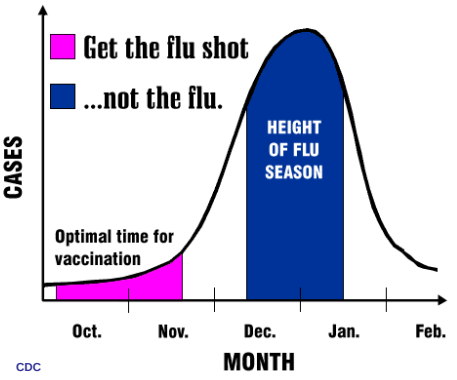
Vaccination

- inactivated
- egg grown
- sub-unit vaccine for children

- reassortant live vaccine approved 2003
  - for healthy persons (those not at risk for complications from influenza infection) ages 5-49 years



https://upload.wikimedia.org/wikipedia/commons/thumb/d/d7/Reassortment.svg/800px-Reassortment.svg.png



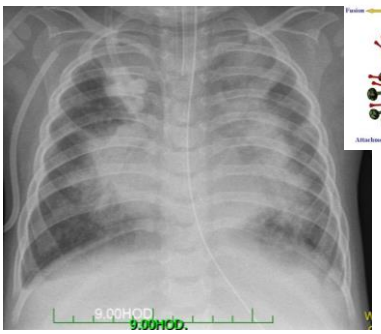
CDC

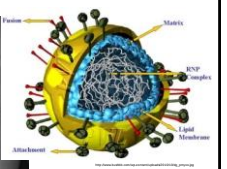
And what about Paramyxoviruses

Paramyxoviridae

Respiratory-syntitial virus

RSV (boy treated for AML)

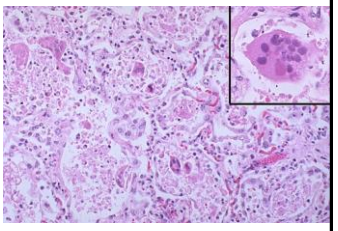




Paramyxoviridae

Pathophysiology

- Negative-strand RNA virus
- Family *Paramyxoviridae*
- RSV season late fall to early spring
- Peak in January/February
- Incubation 4-5 days
- LRI between days 5-7



RSV in a child. Note the giant cells which are part of the viral cytopathic effect. The inset demonstrates a typical giant cell with a round, pink intracytoplasmic inclusion. RSV accounts for many cases of pneumonia in children under 2 years, and can be a cause for death in infants 1 to 6 months of age or older.

http://library.med.utah.edu/WebPath/jpeg3.LURG158.jpg

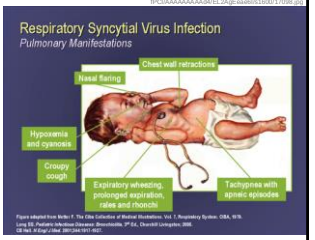
- Most common cause of **bronchiolitis & pneumonia** in children under 1
- 25-40% of children develop bronchiolitis or pneumonia during first RSV infection
- 31/1,000 under 1 yr. are hospitalized with RSV
- 2% will die

Paramyxoviridae

Presentation

- Cold-like sx
- Audible wheezing
- SOB
- Anorexia
- Poor sleeping
- Irritability
- Vomiting
- Choking

http://2.bp.blogspot.com/-1t0cvfUdOkg/T4LLOcIPCI/AAAAAAAAA04/EL2AgEeaeb0/s1600/17098.jpg



Severity

- Inhibition of certain interferons
- Involvement of innate immune system
- Interleukins and chemokines
- Coinfection with other respiratory viruses

http://img.medscape.com/fullsize/migrated/editorial/cmecircle/2008/18697/flash/luedtke/images/slide9.png

Paramyxoviridae

Inhibition of Interferons

- *Interferons believed to have antiviral properties*
- NS1 & NS2 inhibit IFN-alpha/beta
- Inhibition of IFN-gamma causes enhanced IgE production

Innate immune system

- *Activation contributes to inflammation & injury*
- RSV-F glycoprotein may inhibit T-cell activation
- RSV-infected CD8+ cells unable to release IFN-gamma

Interleukins & Chemokines

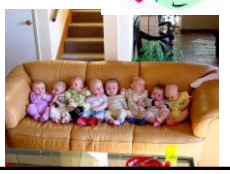
- *Infection induces expression*
- Chemokines mimic RSV glycoproteins
- Recruit monocytes, eosinophils, & neutrophils
- IL-8 levels positively associated with severity

Paramyxoviridae

Coinfection and Risk factors

- Rhinovirus contributes to increased severity in children with bronchiolitis
- Metapneumovirus (hMPV) enhances or mimics symptoms of RSV bronchiolitis
- 70% were coinfected w/ hMPV & required admission to PICU



Paramyxoviridae

Premature Birth

- Likely to have chronic lung disease
- Hypersensitive to stimuli
- Underdeveloped airway & immunity
- Lack adult maternal levels of IgG

Environmental & Demographics

- Male infants
- Age & birth month of infant
- Crowding & day care attendance
- Secondhand smoke

Factors NOT Positively Correlated

- Socioeconomic status
- Malnourishment
- Breastfeeding

Paramyxoviridae

Prophylaxis

- **RSV-IGIV (RespiGam)**
- Children under 24 mo. w/ CHD or less than 35 wks. gestation
- Given IV monthly during RSV season
- Volume overload possible
- Not for infants w/ hemodynamically significant heart disease.

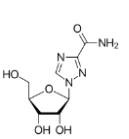
- **Palivizumab (Synagis)** – anti protein F antibody
- Given IM monthly
- Can reduce hospitalization of high risk infants by 45%
- Expensive
- Many providers reluctant to give
- Many parents unaware

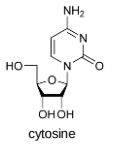
Paramyxoviridae

Treatment

- Mostly symptomatic
- Salbutamol MDI drug of choice
- Also use epinephrine, ipratropium bromide & oral steroids only if hospitalized

- **ribavirine in severely ill patients**





cytosine

Fourth European Conference on Infections in Leukaemia (ECIL-4): Guidelines for Diagnosis and Treatment of Human Respiratory Syncytial Virus, Parainfluenza Virus, Metapneumovirus, Rhinovirus, and Coronavirus

CID 2013

Oral ribavirin for treatment of respiratory syncitial virus and parainfluenza 3 virus infections post allogeneic haematopoietic stem cell transplantation

BMT 2011

p.o. ribavirine 10-30 mg/kg/D in 3 doses

Paramyxoviridae

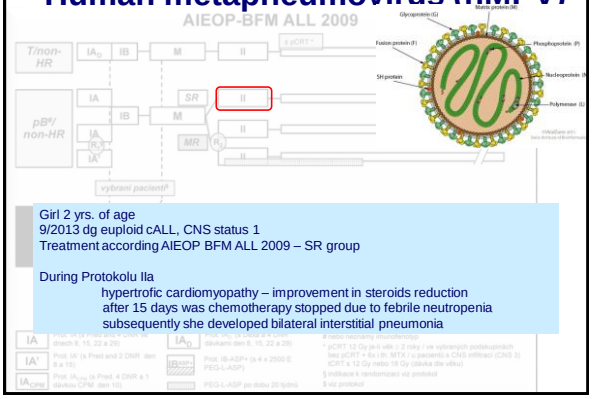
Morbidity & Mortality of RSV

- More likely to visit a specialist
- More likely to use respiratory therapy
- More likely to receive diagnostic or therapeutic procedures
- More likely to be hospitalized again
- Subsequent hospitalization will be 3x as long
- More likely to suffer recurrent infections
- Many have recurrent acute otitis media
- Many likely to be hospitalized with another episode of acute respiratory distress

- Adolescents suffer from allergic asthma, allergic rhinoconjunctivitis, & more sensitive to inhaled allergens
- More likely to have asthma, bronchial reactivity to methacholine, and reduced lung function
- RSV ind. risk factor for reduced FEV% (FEV1/FVC)

Paramyxoviridae

Human metapneumovirus (hMPV)

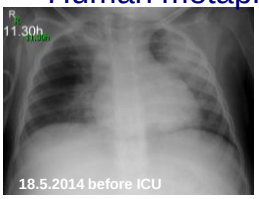


Girl 2 yrs. of age
9/2013 dg euploid cALL, CNS status 1
Treatment according AIEOP BFM ALL 2009 – SR group

During Protokolu IIa
hypertrofic cardiomyopathy – improvement in steroids reduction
after 15 days was chemotherapy stopped due to febrile neutropenia
subsequently she developed bilateral interstitial pneumonia

Paramyxoviridae

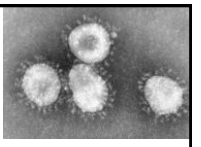
Human metapneumovirus (hMPV)



- 9.5.2014 positive NF swab for hMPV
- Treatment:
  - IVIG (substitution 0.3 g/kg - 4 doses)
  - ribavirine 6 mg/kg á 8 hod p.o. 5 weeks
- Respiratory failure with 8 days of AV (FiO_2 1,0)
- hMPV confirmed for ET tube
- hMPV positivity 4 weeks
- Control CT after 10 days of AV - regression

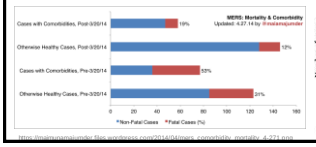
After 4 weeks he finished Protokol IIa.

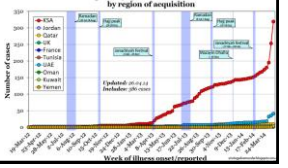
Coronaviruses



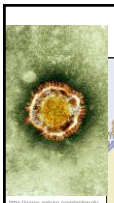
- Coronaviridae
- ss (+) RNA, 26-32 kb genone length (largest RNA)
- first identified in the mid-1960s
  - alpha – HCoV 229E and NL63
  - beta - HCoV OC43, HKU1, SARS-CoV (severe acute respiratory syndrome), and MERS-CoV (Middle East Respiratory Syndrome)
- **SARS**
  - Cellular receptor – ACE2
  - mortality rate – approx. 9.5%
- Incubation period – 2-4 days
- **Treatment symptomatic**

MERS - transmission through camels, their milk and cheese

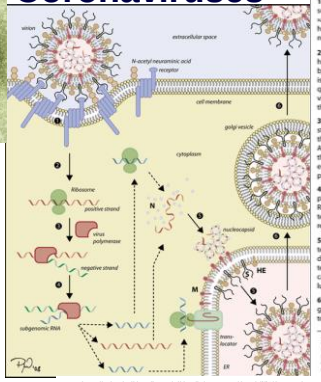




Coronaviruses



http://www.nature.com/polopoly_fs/7.6657.1349167529!/image/11.11513_coronavirus_HPA.jpg_gen/derivatives/landscape_630/11.11513_coronavirus_HPA.jpg



Replication of Coronavirus

1 With their S-protein, coronaviruses bind on cell surface molecules such as the metalloprotease vamino-peptidase N. Viruses, which accessorily have the HE-protein, can also bind on N-acetyl neuraminic acid that serves as a co-receptor.

2 So far, it is not clear whether the virus get into the host cell by fusion of viral and cell membrane or by receptor mediated endocytosis in that the virus is in-corporated via an endosome, which is subsequently acidified by proton pumps. In that case, the virus have to escape destruction and transport to the lysosome.

3 Since coronaviruses have a single positive stranded RNA genome, they can directly produce their proteins and new genomes in the cytoplasm. At first, the virus synthesize its RNA polymerase that only recognizes and produces viral RNAs. This enzyme synthesize the minus strand using the positive strand as template.

4 Subsequently, this negative strand serves as template to transcribe smaller subgenomic positive RNAs which are used to synthesize all other proteins. Furthermore, this negative strand serves for replication of new positive stranded RNA genomes.

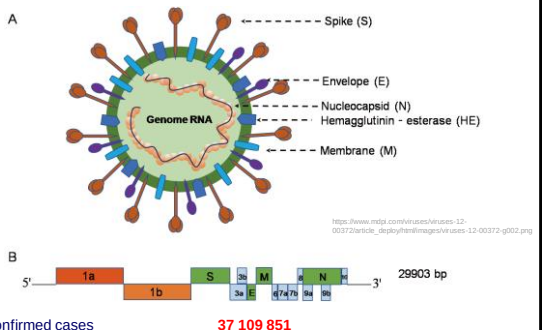
5 The protein N binds genomic RNA and the protein M is integrated into the membrane of the endoplasmatic reticulum (ER) like the envelope proteins S and HE. After binding, assembled nucleocapsids with helical twisted RNA budd into the ER lumen and are encased with its membrane.

6 These viral progeny are finally transported by golgi vesicles to the cell membrane and are exocytosed into the extracellular space.

Not drawn to scale! Not all cellular compartments and enzymes are shown. Colors: positive strand RNA (red), negative strand RNA (green), subgenomic RNAs (blue).
Based on: Lai MMC, Cavanagh D (1997). The molecular biology of coronavirus. Adv Virus Res 48:1–100.

https://upload.wikimedia.org/wikipedia/commons/thumb/4/44/Coronavirus_replication.png/800px-Coronavirus_replication.png

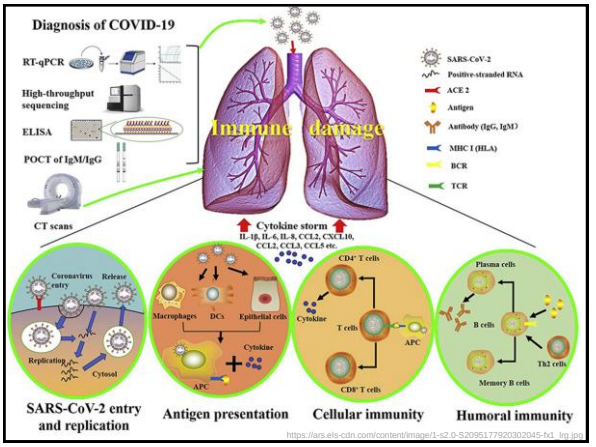
SARS-CoV-2

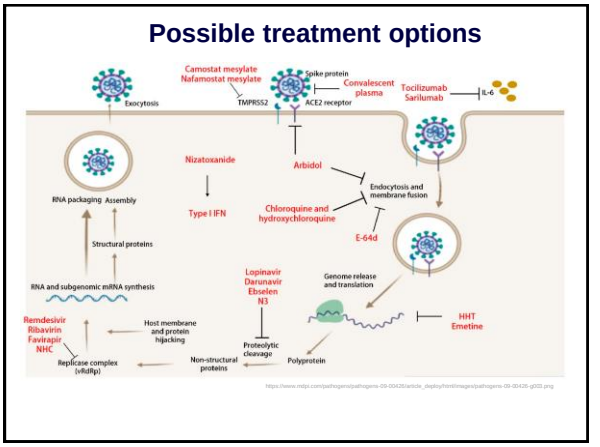


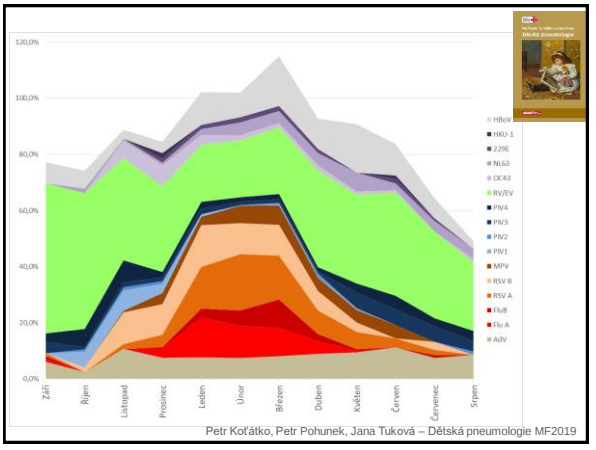
https://www.mdpi.com/viruses/viruses-12-00372/article_deploy/html/images/viruses-12-00372-g002.png

Confirmed cases	37 109 851
Confirmed deaths	1 070 355
Countries, areas or territories with	235

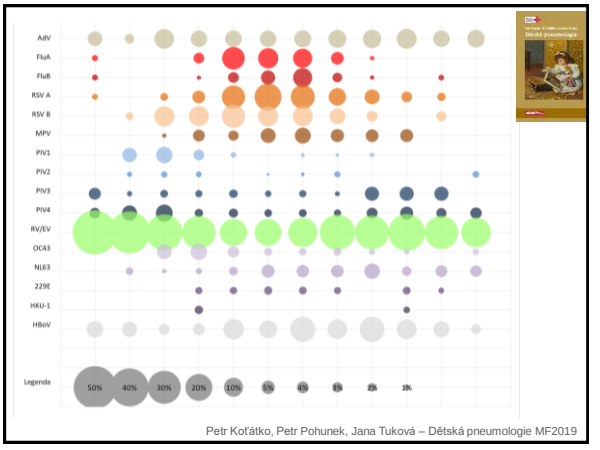
Last update: 11 October 2020, 02:00 CEST



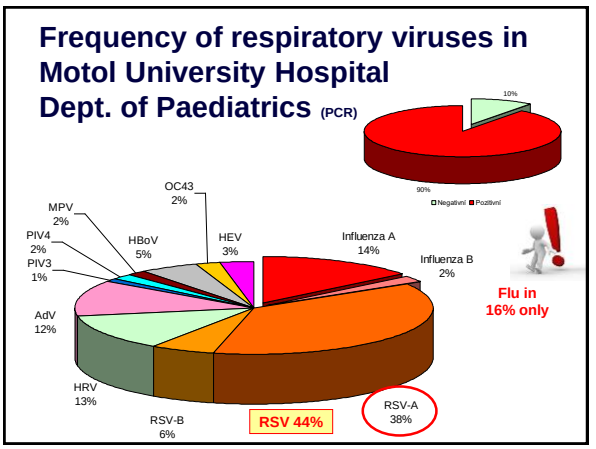


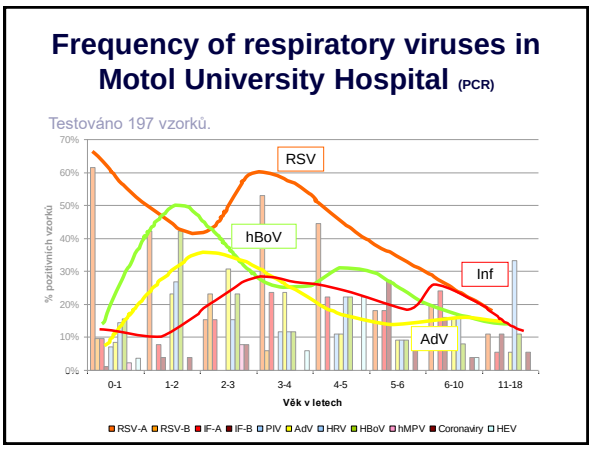


Petr Koťátko, Petr Pohunek, Jana Tuková – Dětská pneumologie MF2019

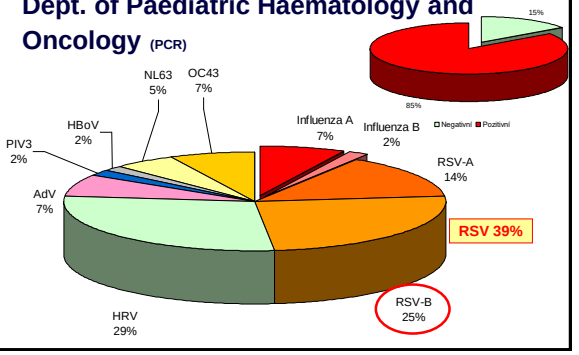


Petr Koťátko, Petr Pohunek, Jana Tuková – Dětská pneumologie MF2019





Frequency of respiratory viruses in Motol University Hospital
Dept. of Paediatric Haematology and Oncology (PCR)



CAVE

Every detection technique has limits!

Even molecular-biological = PCR!

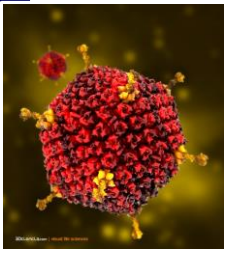
It is true also for commercial kits e.g. There is evidence that Anyplex RV16 detects only **10 out of 60 described serotypes.**

Most frequently detected,

but not the only!!!!

PCR negativity does not

neccesary omits AdV infection.



REVIEW ARTICLE

258 • CID 2013:56 (15 January) • Hirsch et al

Fourth European Conference on Infections in Leukaemia (ECIL-4): Guidelines for Diagnosis and Treatment of Human Respiratory Syncytial Virus, Parainfluenza Virus, Metapneumovirus, Rhinovirus, and Coronavirus

Hans H. Hirsch,[1,2] Rodrigo Martino,[3] Katherine N. Ward,[4] Michael Boeckh,[5] Hermann Einsele,[6] and Per Ljungman[7,8]

http://www.ebmt.org/Contents/Resources/Library/ECIL/Pages/ECIL.aspx

Viral exanthematic diseases

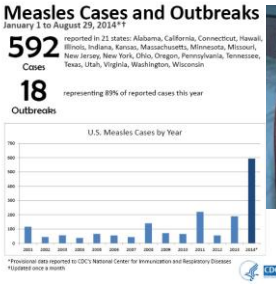
Childhood exanthema diseases

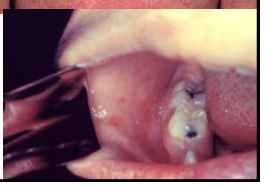
Classical name	„systematic exant. name“	Pathogen
Measles (rubeola)	1st childhood disease	morbillivirus
Scarlet fever	2nd childhood disease	Streptococcus pyogenes
Rubella (German measles)	3rd childhood disease	Rubivirus
Filatov-Duke‘s disease (pseudoscarlantina)	4th childhood disease	Coxackie and Echoviruses
Erythema infectiosum	5th childhood disease	Parvovirus B19
Exanthema subitum – Roseola infantum	6th childhood disease	HHV-6 and HHV-7

Chicken pox - VZV

Paramyxoviridae

Measles





Estimated cases – 20,000,000 / year.
Estimated kills - 164,000 people in world/year.

Paramyxoviridae

Measles

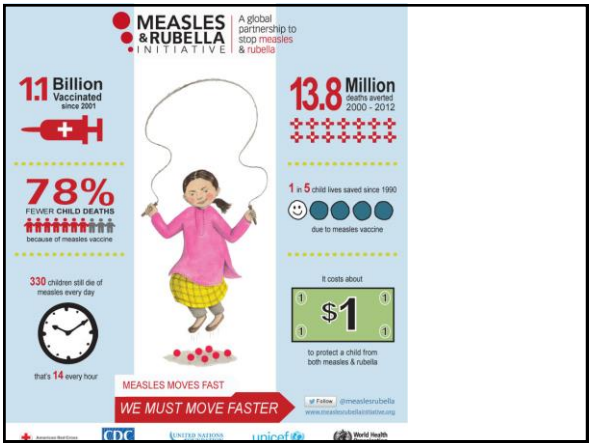
- Respiratory disease caused by a morbillivirus
- Measles virus normally grows in the cells that line the back of the throat and lungs
- ss (-) RNA virus of genome length 15-16 kb, coding 8 proteins
- spherical symetry of capsid and diameter of 100-300 nm
- incubation period 8-12 days

Symptoms
Measles causes fever, runny nose, cough and a rash all over the body. Rash starts at head and neck and spreads from this areas to whole body.

Complications
About 1 / 10 children gets an ear infection, and up to 1 out of 20 gets pneumonia. About 1 out of 1,000 gets encephalitis, and 1-2 out of 1,000 die.

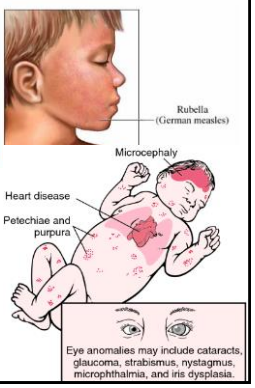
Transmission
Spreads through the air by breathing, coughing or sneezing.
It is so contagious that any child who is exposed to it and is not immune will probably get the disease.

There is vaccination against measles.



Togaviridae

Rubella - German measles



- Rubivirus (RNA)
- incubation period avr. 18 days (12-23)
- viraemia 5th-7th day after exposition with subsequent speading to the organs

Togaviridae

Rubella - German measles

The infection is usually mild with fever and rash. In pregnancy the virus can cause serious birth defects.

Symptoms: in children: Rash that starts on the face and spreads to the rest of the body, Low fever. Usually a mild disease
These symptoms last 2 or 3 days.
Older children and adults: swollen glands and symptoms cold-like sy. before the rash. Aching joints occur in many cases, especially among young women.
About ½ of the people do not have symptoms.
In rare cases, serious problems can occur. These include brain infections and bleeding problems.

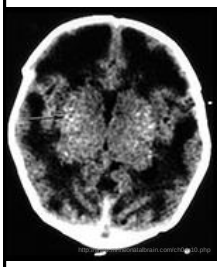
In pregnancy: miscarriage or birth defects like deafness, intellectual disability, and heart defects. 85% of babies born to mothers who had rubella in the first 3 months of her pregnancy will have a birth defect.
Spreading: through coughs or sneezes; most contagious when the person has a rash. But it can spread up to 7 days before the rash appears. People without symptoms can still spread rubella.

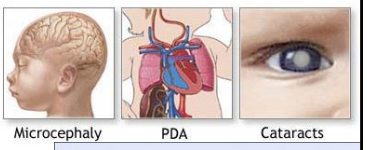
The MMR vaccine protects against rubella.

Togaviridae

Rubella - German measles

Rubella syndrome





Box 1: Clinical features of congenital rubella syndrome

Classic triad

- *Congenital heart disease* (e.g., patent ductus arteriosis, pulmonary artery stenosis, pulmonary valvular stenosis)
- *Ocular defects* (e.g., congenital cataracts, microphthalmos, pigmentary retinopathy, congenital glaucoma)
- *Hearing loss*

Congenital rubella syndrome is usually associated with a failure to thrive and developmental delay as well as microcephaly. Other common presentations at birth include:

- purpuric rash
- hepatosplenomegaly
- meningoencephalitis
- radiolucent bone
- hepatitis
- thrombocytopenia

Infection between 8th-10th week of gestation leads to development of congenital rubella syndrome in 90%.

Congenital infections with Venezuelan Equine Encephalitis Virus are symptomatically similar.

Rubella and measles

Details e.g. also in:

http://ecdc.europa.eu/en/publications/Publications/systematic-review-incubation-period-shedding-children.pdf

Parvovirus B19

Described in Australia in 1975 by Yvonne Cossart, in microtitration plate „B19".

Proliferation in erythroid cells of bone marrow (dysregulation of cell cycle through NS1 protein.

Transmission by droplets, mainly. Incubation: 2 weeks (4-28 day) lasting for a week.

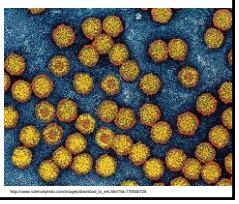
Erythema infectiosum („slapped cheek") – „Fifths disease".

Teenage - "Papular Purpuric Gloves and Socks Syndrome".

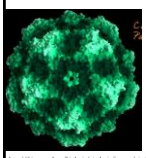
Adults – urticas; Pregnant hydrops foetalis

Immunosupressed patients - „pure red cell aplasia".

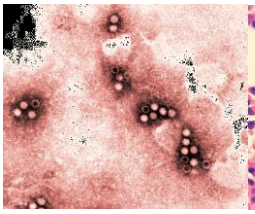
Described possible related complication of B19 infection is myocarditis.

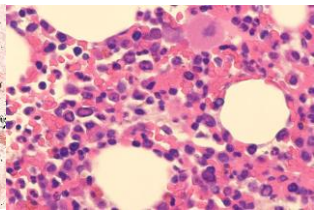


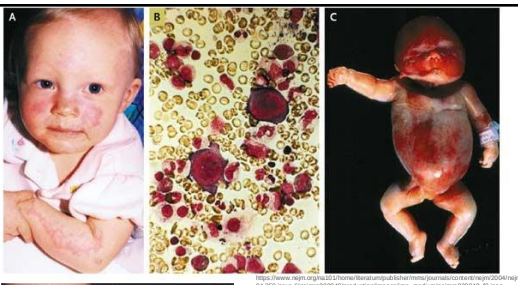
Parvovirus B19

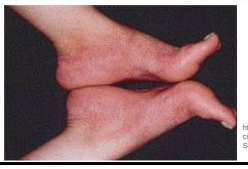


- small ss DNA +/-
- Capsid 20-26 nm, genome: 5 kbp
- E.g. Aplastic anaemia…









Human herpesvirus 6

Previously two variants of HHV-6.
Recently 2 distinct viral species

HHV-6 A

Unknown

„Orphan virus“

HHV-6 B

Immunocompetent host

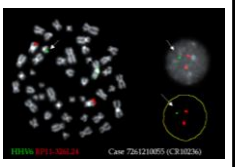
- **Sixth disease**
- **Febrile seizures**
- **Encephalitis**

Immunocompromised host

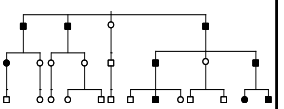
- **Encephalitis**
- **Myelosupression**
- Hepatitis
- Pneumonitis
- Pericarditis
- **Delayed engraftment after HSCT**

Chromosomally integrated HHV-6 (CI-HHV-6)

- **Viral DNA integrated into human chromosomes**
  - **Inherited from parents to child**
  - **Viral DNA is present in every body cell** (e.g.hair roots, nails)
  - **Ratio of viral DNA : human DNA = 1:1**
- **Described frequency in population between 0.2-2.9%** (Tanaka-Taya 2004, Ward 2007)
- **Both variants (A or B) integrates**
- **No clear observed reactivation CI-HHV-6 to active infection in vivo**
- **In vitro reactivations are doubtful**

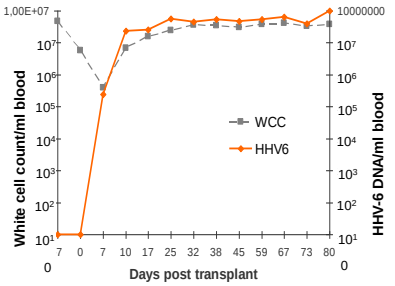


HHV-6 integration at 22q13.3 control probe on 9q34.4



HHV6 DNA in blood after HSCT

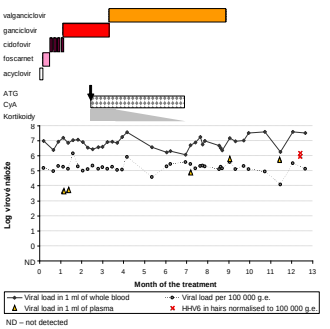
donor with Ci-HHV-6



Clark et al., JID 2006

Patient 2

Chromosomally integrated HHV-6 (Ci-HHV-6)



Patient with SAA

50 years

After start of the IS therapy– partial response only

Dependent of thrombocyte infusion

G-CSF therapy

Died due to peracute sepsis of *St. aureus*.

Detection of high HHV-6 DNA quantity is NOT NECESSARY an active infection.

Detection in hair, or nails detects Ci-HHV-6 safely.

And now what?

DNA viruses

ds DNA

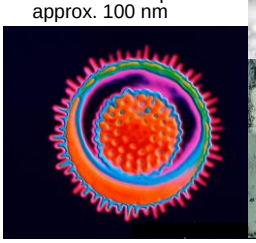
- *Herpesviridae* → α HSV, VZV; β CMV, HHV-6 a 7; γ EBV, HHV8
- *Adenoviridae* → Adenoviruses (group A-F)
- *Polyomaviridae* → BKV, JCV, WUV, KIV, SV40...
- *Papillomaviridae* → Papillomaviry
- *Poxviridae* → Variolla virus, vaccinia, molluscum contagiosum
- *Hepadna* → HBV

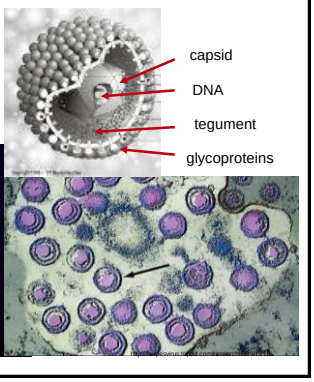
ss DNA

- *Parvoviridae* → Parvovirus B19, lidský bocavirus (HBoV)
- *Anelloviridae* → TTV, TTMV, TTMDV

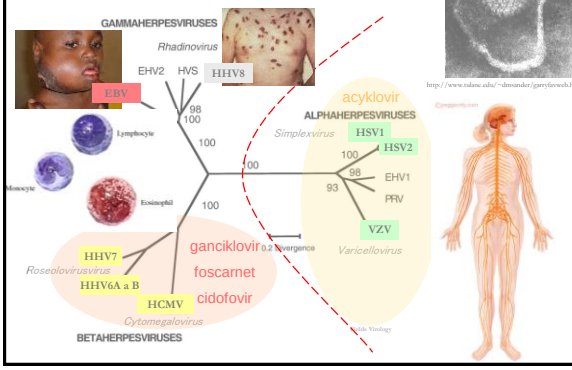
Herpesviruses

- Enveloped ds DNA viruses
- Genome of length 125-240 kb
- Icosahedral capsid
- Diametre of capsid of approx. 100 nm



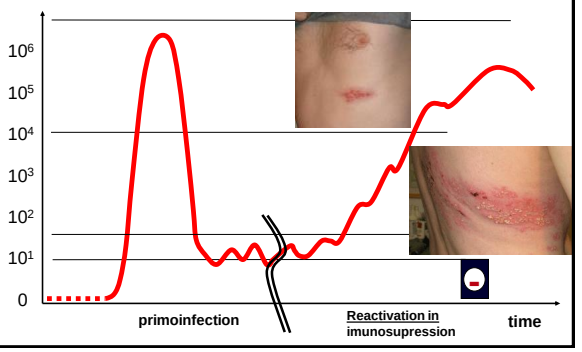


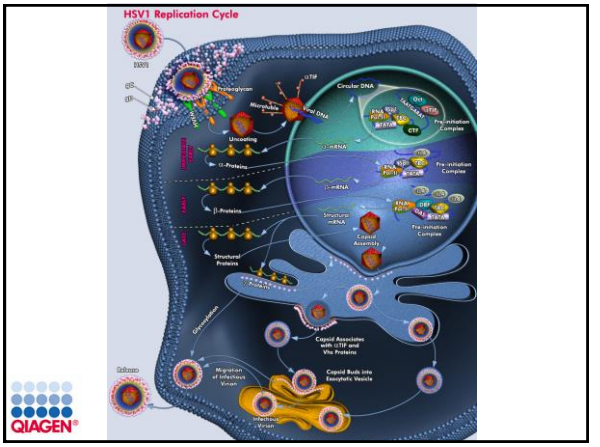
Taxonomy

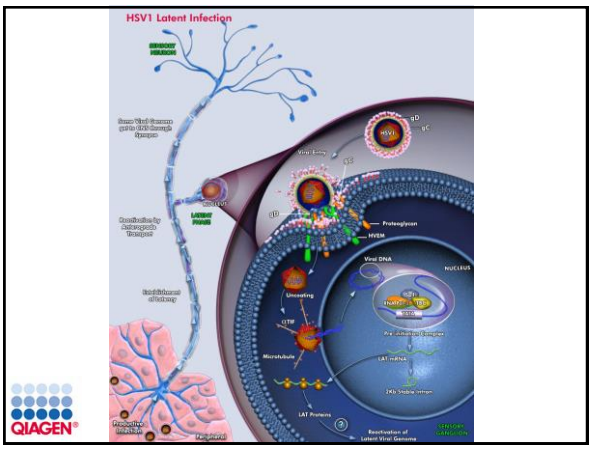


Latency with posibility of reactivation

Transmission – by body fluids (saliva, urine, breast milk, blood, …)





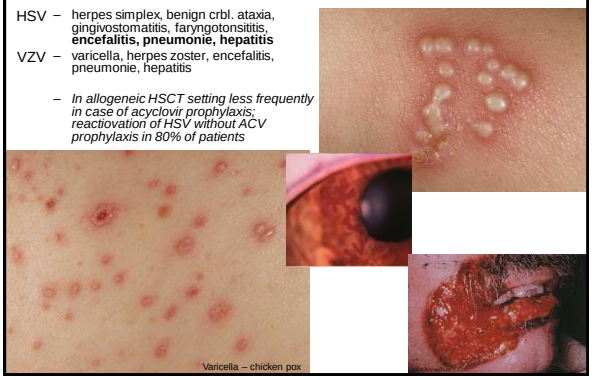


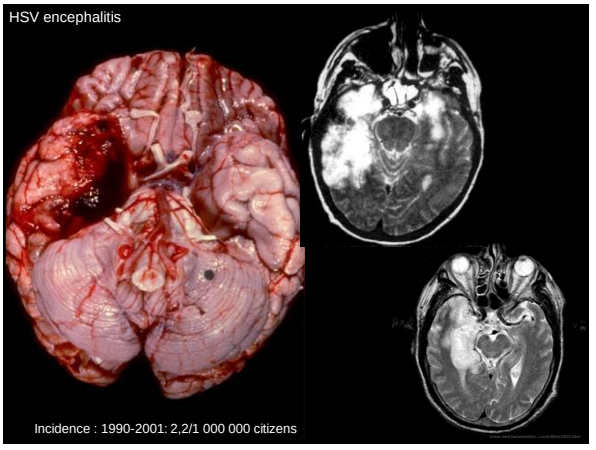
Pathological impact of HSV and VZV

HSV – herpes simplex, benign crbl. ataxia, gingivostomatitis, faryngotonsilitis, **encefalitis, pneumonie, hepatitis**

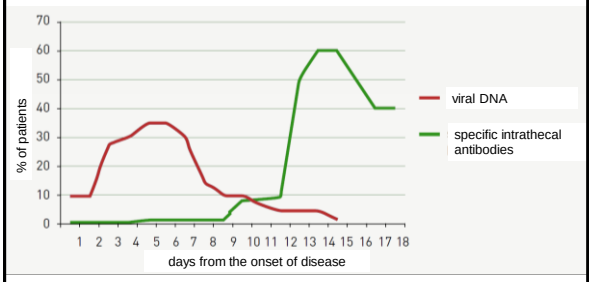
VZV – varicella, herpes zoster, encefalitis, pneumonie, hepatitis

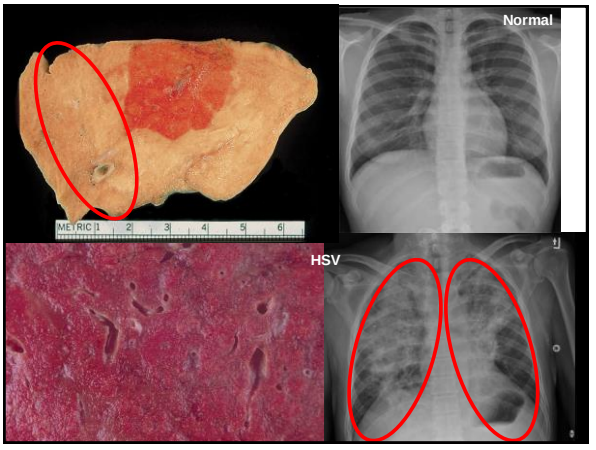
– *In allogeneic HSCT setting less frequently in case of acyclovir prophylaxis; reactiovation of HSV without ACV prophylaxis in 80% of patients*





Antibody response to viral infection and detection of virus in CSF

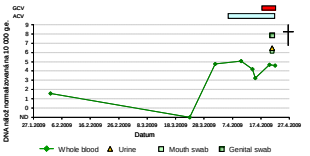




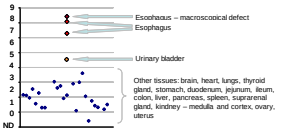
Different impact and destruction in different organs

HSV (girl treated for ALL)

HSV pneumonia

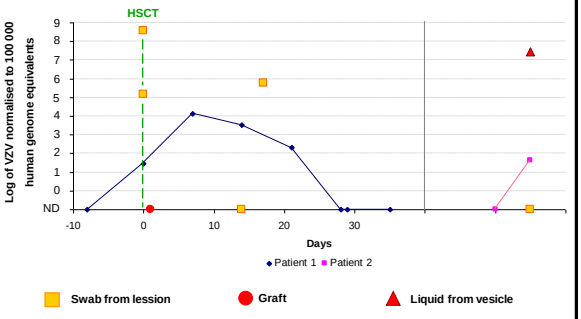


HSV quantity detected in the tissue at the authopsy

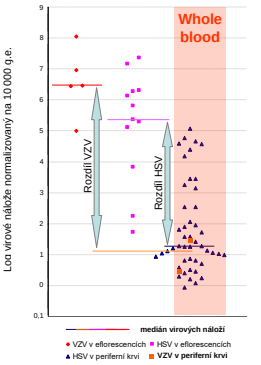


Difference in materials

VZV – chicken pox at D+0



Source for viral detection



January 2004 to August 2011

- **HSV in 735 samples from 266 patients**
  VZV in 587 samples from 148 patients
- **569 whole blood samples**
- **43 swab samples from skin, mucousal tissue and aspirates from vesicles (from 15 p.)**
- **227 samples from other biological materials (stool, urine, CSF, tissues)**

HSV detected

- **in 12 samples from eflorescence from 9 pts; medián of quantity 439,465 NVC**
  (range 53-23,380,000 NVC)
- **6 pts in whole blood samples; median of viral load 18,7 NVC**
  (range 0.88 – 1,216,650 NVC)
- **4 in stool with median 53,662 NVC**
  (range 1,248-900,000 NVC)

VZV detected

- **in 8 samples from skin eruption from 5 pts; median of quantity 2,856,124 NVC**
  (range 13,939-114,464,380 NVC)
- **in 2 pts. In whole blood (quantity 30 and 2,9 NVC)**

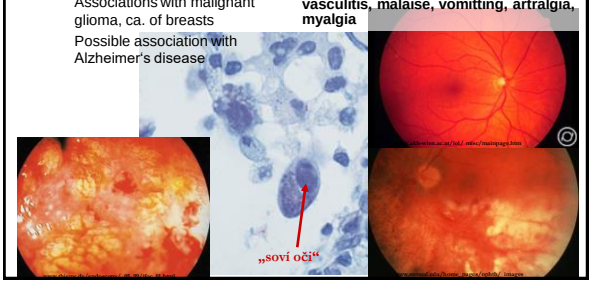
Pathological impact of CMV

In immunocompetent

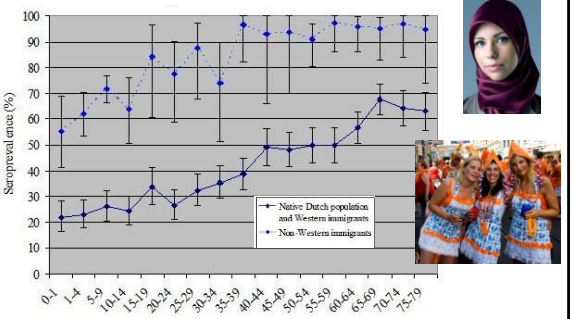
- Asymptomatic in 95% of children
- mononukleosis like sy.
- In pregnant woman teratogenic
- Associations with malignant glioma, ca. of breasts
- Possible association with Alzheimer's disease

In immunocompromised mainly

trombocytopenia, pneumonitis, hepatitis, encefalitis, retinitis, colitis, esofagitis, pankreatitis, vasculitis, malaise, vomitting, artralgia, myalgia



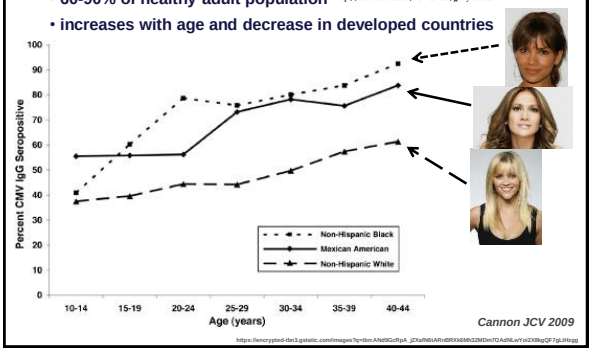
CMV seroprevalence



Korndewal et al. European Society for Paediatric Infectious Disease 2012

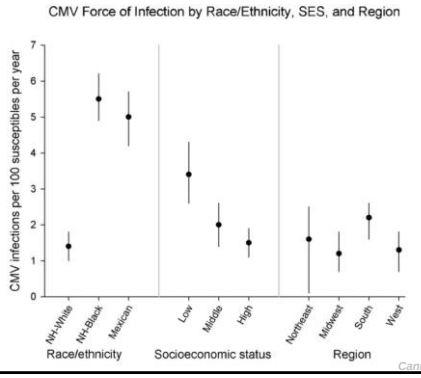
CMV seroprevalence

- **60-90% of healthy adult population** http://www.tulane.edu/~dmsander/garryfavweb.html
- **increases with age and decrease in developed countries**



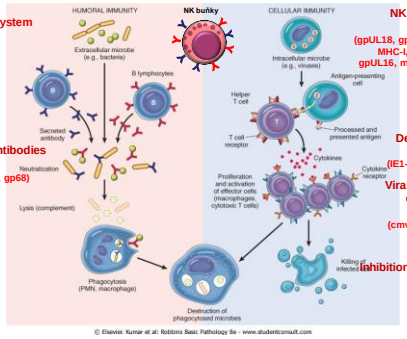
Cannon JCV 2009

Incidence of CMV primoinfection



Cannon JCV 2009

How CMV manipulates with immunity?



Complement system inhibition (?)

Inhibition of antibodies by FcR homolog (gp34, gp68)

NK cells function inhibition (gpUL18, gpUL142-homolog MHC-I, pp65, gpUL141, gpUL16, miR-UL112, UL40)

Decrease of INF production (IE1-p72, IE86, pp65)

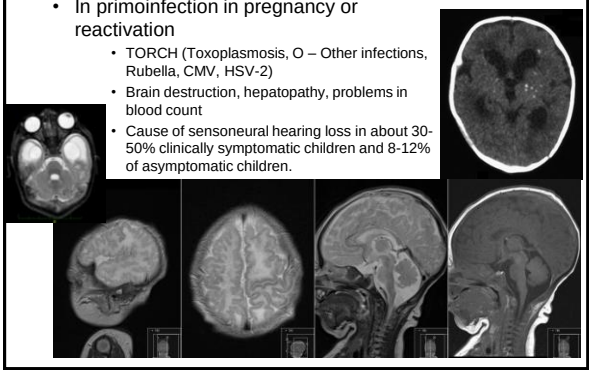
Viral homologs of cytokines and chemokines (cmvIL-10, vCXCL-1, pUL147, pUL128-131)

Inhibition of apoptosis (vICA, vMIA)

Inhibition of expression and function of MHC-I and II (gpUS2, gpUS3, gpUS6, gpUS8, gpUS10, gpUS11, gp34, gp40,gp48, pp65)

Teratogenic impact of CMV

- In primoinfection in pregnancy or reactivation
  - TORCH (Toxoplasmosis, O – Other infections, Rubella, CMV, HSV-2)
  - Brain destruction, hepatopathy, problems in blood count
  - Cause of sensoneural hearing loss in about 30-50% clinically symptomatic children and 8-12% of asymptomatic children.



Symptoms and impact of cCMV

Placental infection

– swalling of the placenta – worse difussion characteristics

- smaller cotymedon development – smaller placental surface

IUGR

Fetal infection

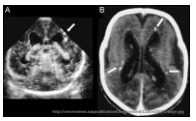
- bone marrow supression **petechia, „blueberry muffin baby“**
- CMV end-organ infection
- vasculitis – especially eyes and a CNS

Neurologic problems/seisures

Brain calcification/ cavity

CMV excretion to urine

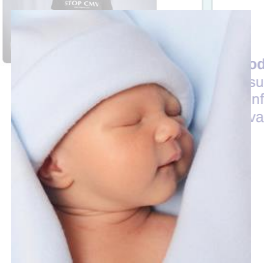
Premature delivery



Symptoms and impact of cCMV

Asymptomatic

90% of children with cCMV



Symptomatic

Congenital CMV infection (cCMV)

According to CDC

Symptoms of congenital CMV at delivery

Premature birth
Hepatopathy
Pulmonary signs
Splenomegaly
IUGR
Neurological seizures

Long term effects of cCMV

Sensoneural hearing loss (SNHL)
Visual loss
Mental disorder
Mikrocephaly
Motorical problems (coordination) ce)
Neurological seizures (epilepsy)
Rarely death

Blueberry muffin baby characterized by purpura as a sign of extramedullary hematopoesis.

Symptoms and impact of cCMV

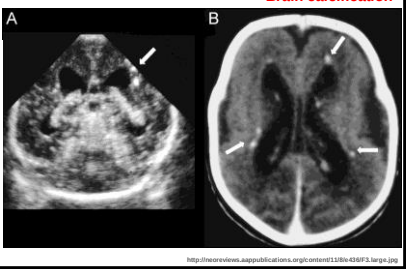
Transient Outcomes

- Hepatomegaly
- Splenomegaly
- Jaundice
- Petechia and purpura
- Pneumonitis
- Fetal growth retardation
- Seizures

Permanent Outcomes

- Microcephaly
- Vision loss
- Hearing loss
- Mental retardation
- Motor disabilities
- Seizures
- Death

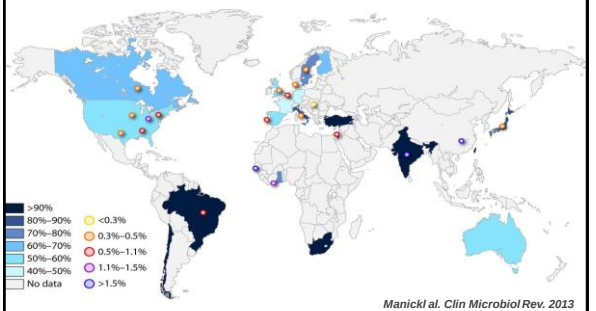
Brain calcification



http://neoreviews.aappublications.org/content/11/8/e436/F3.large.jpg

What is the frequency of cCMV?

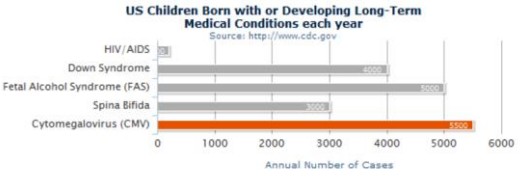
There is 370,000 children born every day in the world, representing 134 millions/year.
Average frequency approx. 1,5% of living birth – 2.01 millions of children with cCMV/year.
In Europe and Czech Republic is estimated frequence 0.5-1% cCMV of living neoborns.

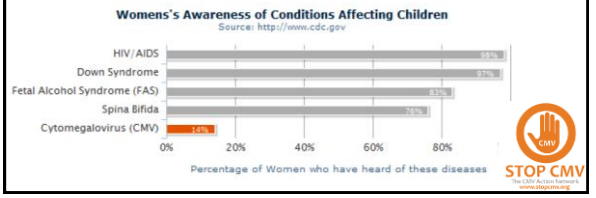


Manickl al. Clin Microbiol Rev. 2013

http://cmr.asm.org/content/26/1/86/F7.large.jpg

What is a knowlegde about cCMV and its impacts?





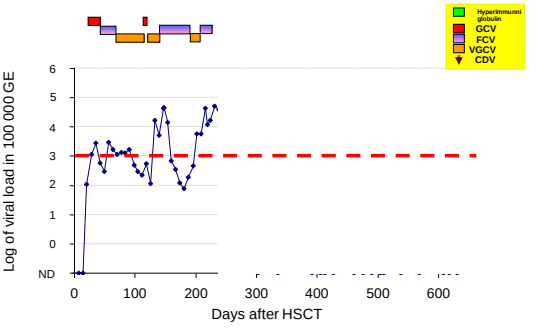
Patient 1

Girl, 16.5 yrs of age at HSCT
Allogeneic HSCT for AML M2 (AML1/ETO+) in 2nd CR
MMUD – 7/10
Conditioning: Busulphan, Cyclofosfamid, Melphalan, ATG
Graft: Periferal stem cells
CD34+: 11,12 x 10^6 /kg; CD3+: 302,1 x 10^6 /kg; NC: 12,09x10^8 /kg
GvHD profylaxis: MTX and CsA
CMV status donor/recipient: D-/R+

Non-CMV complications:
D+16 haemorrhagic cystitis –hyperhydration
D+61 –GvHD grade II (skin and GIT)
therapy : steroids 1 mg/kg
D+377 – Herpes zoster – acyclovir treatment
D+440 – Laser coagulation of retinal bleeding
(not proven, suspected, active CMV retinitis)

Patient 1

D+ 29 – first CMV treatment



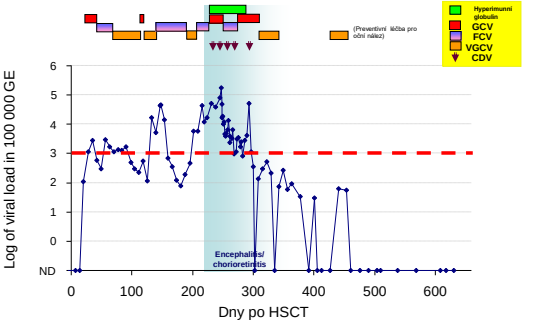
Patient 1

D+230 – during foscarnet treatment patient presented diplopy, headache, vomitting and sleepness.
CMV detected in CSF (approx. 2 600 000 copies / ml) and increase of viral load in peripheral blood.

Results confirmed **encephalitis and** bilateral **chorioretinitis**.

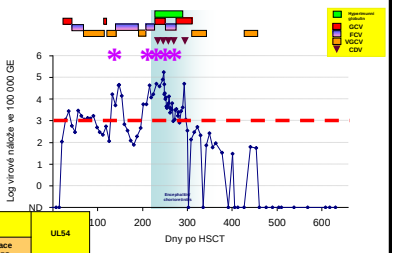
1 – retinal fibrotisation
2 – intraretinal bleeding
3- epiretinal pseudomembrane

Patient 1



Patient 1

Ganciclovir resistance

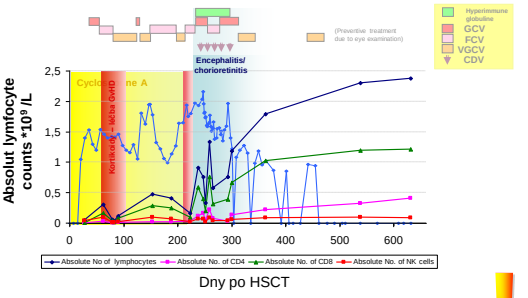


Den	Materiál	Gen UL97		UL54
		Mutace A594V	Mutace L595S	
146	Krev	WT+mutation	WT+mutation	ND
209	Krev	WT	Mutatiom	ND
231	Krev	WT	Mutatiom	ND
234	CSF	WT+mutation	Mutatiom	ND
238	Krev	ND	ND	Mutatiom
247	Krev	ND	ND	Mutatiom
248	Krev	WT+mutation	Mutatiom	ND
276	Plazma	WT	Mutatiom	ND

Possibility of resistance mutant detection is in case of long-lasted treatment usefull.

Patient 1

Lymfocyte counts



Even a short term steroid treatment leads to decrease of the lymfocyte count neccessary for infection control

Patient 1

Outcome

- Recently the patient is regularly controlled by ophtalmologests. Visus in one eye is very limited, however the second eye is healthy. In boths eyes there is limitation of peripheral visus.
- There are no signs of relaps of the primary disease, GvHD and other infections including CMV.

1 – retinal fibrotization

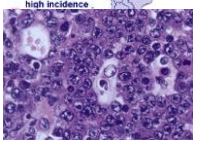
EBV discovery

surgeon

1958
„A sarcoma involving the jaws of African children." British Journal of Surgery

1st described human oncogenic virus

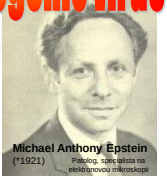
Burkitt's lymphoma high incidence



1961
„The Commonest Children's Cancer in Tropical Africa — A Hitherto Unrecognised Syndrome."

1963 - 1. kultivace viru

1964 – Publikováno v Lancet:
„Cultivation in vitro of human lymphoblasts from Burkitt's malignant Lymphoma"



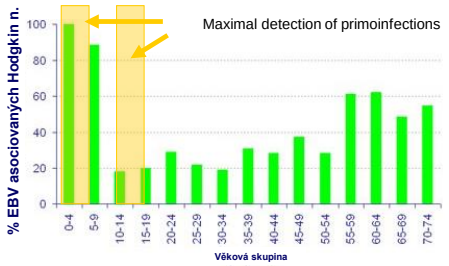
Michael Anthony Epstein (*1921)
Patolog. specialista na elektronovou mikroskopii

Yvonne M. Barr (*1932)
Bert Geoffrey Achong (1928-1996)

Transmission and epidemiology

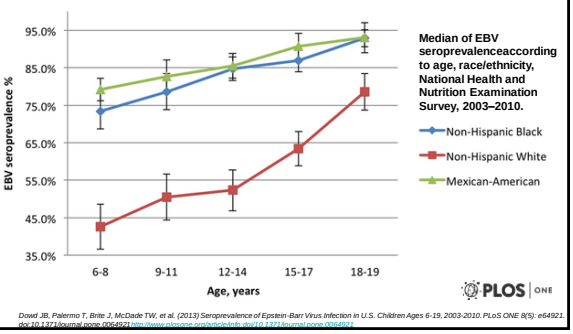
- **Transmission through saliva by oral route**
- **80 - 90% adult population is seropositive**

(in developing countries, it is 90% of children older 2 yrs)



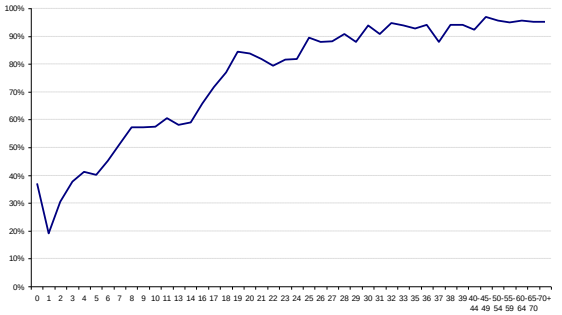
Transmission and epidemiology

- **Transmission through saliva and oral route**
- (permissive cells: B lymfocytes and epithelial cells)
- **80 - 90% of adults population is seropositive**

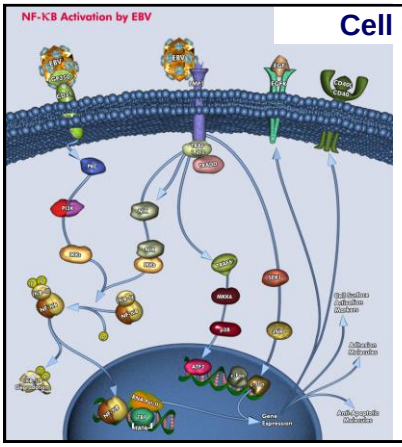


Dowd JB, Palermo T, Brite J, McDade TW, et al. (2013) Seroprevalence of Epstein-Barr Virus Infection in U.S. Children Ages 6-19, 2003-2010. PLoS ONE 8(5): e64921. doi:10.1371/journal.pone.0064921 http://www.plosone.org/article/info:doi/10.1371/journal.pone.0064921

Transmission and epidemiology of EBV in Motol UH



Cell entrance



gp350/220

Binds to:

CD21(CR-2)

HLA II

Permissive cells

B lymfocytes

Epithelial cells

How EBV manipulates the immunity /prolipheration?

EBNA-1	Sequence-specific DNA-binding protein to EBV element; sequence-nonspecific chromosome association protein; transactivator of viral latent genes and host genes; responsible for episome replication, segregation and persistence of viral genome; involved in p53 degradation and oncogenesis
EBNA-LP	Transcriptional coactivator of EBNA-2-dependent viral and cellular gene transcription; primarily indirectly associates with host DNA sites located at or near the transcriptional start; associates with cellular transcriptional (co)factors and EBNA-2; dismisses repressor complex from promoter or enhancer sites; is essential for EBV-mediated B-cell transformation
EBNA-2	Together with EBNA-LP cooperatively activates viral and cellular gene transcription for transformation; primarily indirectly associates with host DNA sites located at the enhancer or intergenic region; associates with cellular transcriptional (co)factors and EBNA-LP; is critical for EBV-mediated B-cell transformation
EBNA-3A	A coactivator of EBNA-2, EBNA-3A and EBNA-3C associations with RBPJ inhibit RBPJ recruitments to DNA; downregulate cMyc transcription and block EBNA-2 activation effects; and induce CDKN2 and chemokines. Induces G1 arrests, which is essential for EBV-mediated B-cell transformation
EBNA-3B	A coactivator of EBNA-2; dispensable for B-cell transformation; viral tumor suppressor; and upregulates CXCL10. EBNA-3B-knockout induces DLBCL-like tumors
EBNA-3C	Coactivates with EBNA-2 host *CXCR4* and *CXCL12* genes; induces CDKN2, chemokines and aurora kinase B; mediates RB degradation; attenuates H2AX expression and overcomes EBV-infection-mediated DNA damage response; promotes cell proliferation; induces G1 arrests; essential for EBV-mediated B-cell transformation
LMP-1	Mimics the constitutively active form of CD40, a major EBV-encoded oncogene; activates NF-κB, JNK and p38 pathways; is critical for EBV-mediated B-cell transformation, a major EBV-encoded oncogene; activates NF-κB, JNK and p38 pathways; and induces EMT of NPC and acquisition of CSC-like properties
LMP-2A	Mimics constitutively active, antigen-independent BCR signaling through constitutive activation of the ERK/MAPK pathway224; blocks antigen-dependent BCR signaling; induces B-cell lymphoma in transgenic condition; is important but not essential for *in vitro* primary B-lymphocyte growth transformation; rescues the LMP-1-generated impairment in germinal center in the response to antigen in animals; confers resting B cells sensitive to NF-κB inhibition and apoptosis; suppresses differentiation and promotes epithelial cell spreading and motility in epithelial cells, and enriches cancer stem cell-like population
EBER	Most abundant EBV-encoded noncoding RNAs; augments colony formation and induces growth; confers cells resistance to PKR-dependent apoptosis; induces cytokines and modulates innate immune response; binds to La, PKR, L22, PRR and RIG-I; and EBER-mediated RIG-I activation likely contributes to EBV oncogenesis. EBER blockades of PKR-mediated phosphorylation of eIF2α results in blockage of eIF2α-mediated inhibition of protein synthesis and resistance to IFNα-induced apoptosis
miRNAs	Transcribed from BART and BHRF1; validated targets include Bim, BRUCE, CXCL11, DICER1, PUMA; has a role in sustaining latently infected cells. BHRF1 miRNA and BART miRNAs interfere with apoptosis. The miR-BART15-3p promoted apoptosis 331

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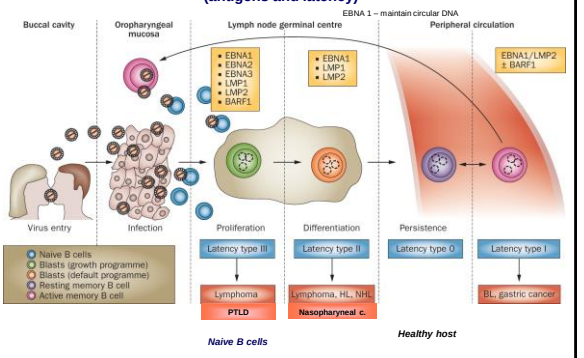
How EBV manipulates the immunity

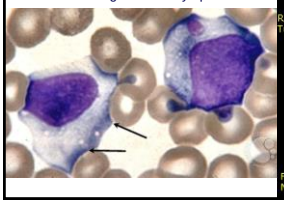
(antigens and latency)

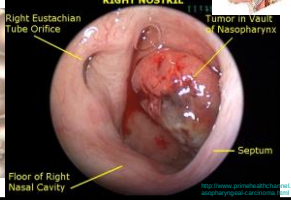


Ballard, C. M. et al. (2012) T-cell therapy in the treatment of post-transplant lymphoproliferative disease. *Nat. Rev. Clin. Oncol.* doi:10.1038/nrclinonc.2012.111

Patological activities of EBV

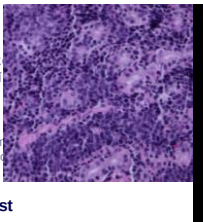
- **Immunocompetent host**
  - Infectious mononucleosis
  - Chronic active EBV infection
  - X-binded lymfoprolipherative disease
  - Malignant diseases
    - Hodgkin disease
    - Burkitt's lymphoma
    - non-Hodgkin T/NK lymphoma
    - Nasopharyngeal carcinoma
    - Gastric carcinoma
    - Angioblastic T lymphoma

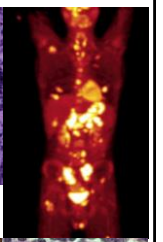


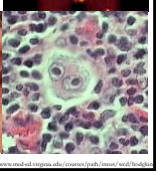


Patological activities of EBV

- **Imunokompetentní hostitel**
  - Infekční mononukleóza
  - Chronická aktivní EBV infek
  - X-vázaná lymfoproliferativní
  - Maligní onemocnění
    - Hodgkinova nemoc
    - Burkittův lymfom
    - non-Hodgkinský T/NK ly
    - Nasopharyngeální karcin
    - Karcinom žaludku
    - Angioblastický T lymfom
- **Immunocompromised host**
  - Hairy leukoplaky
  - Above listed malignant diseases
  - Post-transplant lymfoproliferative disease (EBV-LPD)
  - Encefalitis/myelitis
  - Pneumonie
  - Hepatopathy/hepatitis







Infectious mononucleosis

- „Kissing disease“
- Proliferation affects spleen, liver and lymph nodes
- Tiredness lasting for weeks, increased temperature and fevers (often approx. 39 ° C), pharyngitis and swelling of the lymph nodes (submandibular and cervical); hepatosplenomegaly, hepatopathy, swelling of the eye lashes and face, malaise
- Inkubation period 4-6 weeks
- At the beginning seems like „tonsilitis“
- Transmission by saliva
- Treatment approx. 6 months Relax and diet (2-3 months); Subsequently it is neccesary to have some relax in physical activity

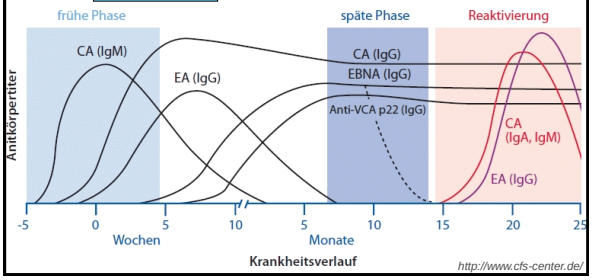
http://home.teleport.com/~bobbi/InfectiousMononucleosis.htm

Diagnostics

Basic diagnosis of EBV is indirect – serological.

Early phase | Post-acute phase | Late phase | Reactivation

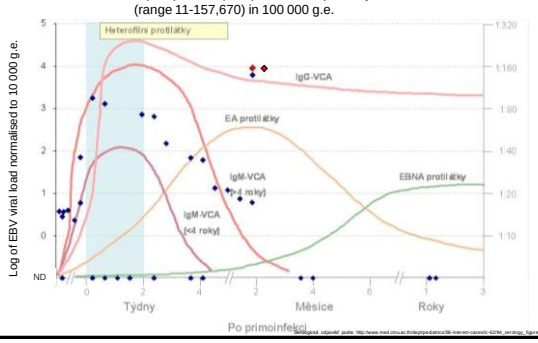
Heterofile antibodies | Paul-Bunnelova reaction



http://www.cfs-center.de/

Viral load in patients with dg. B27 - IM

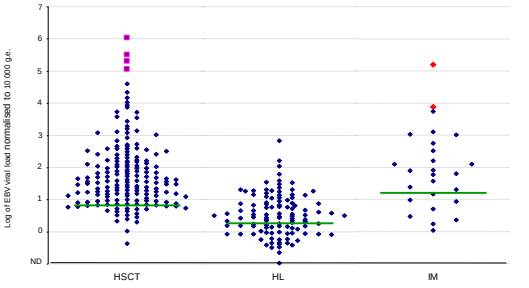
- Positive – 26 patients (62%)
  - 50 samples positive (65%); median of positivity 110 (range 11-157,670) in 100 000 g.e.



Obrázek odpovídá podle: http://www.med.cmu.ac.th/dept/pediatrics/08-interest-cases/ic-62/IM_serology_figure2-1.jpg

Direct detection - PCR

- Detection in peripheral blood (plasma, whole blood), possibly in the tissue
- In HL and IM, EBV is detected in peripheral blood in low quantity.
- Median of detected quantity in whole blood increased from HL→ HSCT → IM



Chronic active EBV infection

Infected T lymfocytes and NK cells

Signs often connected with prolonged presence of interferons in the organism.

Diagnostic criteria of a case definition for SCAEBV [15]

Category	Criteria
Clinical	Intermittent fever, lymphadenopathy, and hepatosplenomegaly.
Hematologic	Anemia, thrombocytopenia, lymphocytopenia or lymphocytosis, neutropenia, and polyclonal gammopathy.
Virological	Elevated antibody titers and positivity for antibodies to EBV-related antigens (VCA IgG, ≥5120; VCA IgA, positive; EA [D] IgG, ≥640; EA [D] IgA, positive; and EA [D] and EA [R] IgG, ≥640) and/or detection of EBV genomes in affected tissues.
Other	Chronic illness that cannot be explained by other known disease processes.

F. Sánchez et al. / Annals of Diagnostic Pathology 12 (2008) 368–371

Chronic active EBV infection

	T-cell type (n = 16)	NK-cell type (n = 12)	P
Symptoms			
Fever, > 1 d/wk (%)	67	25	.04
HMB (%)	13	75	.002
Splenomegaly (%)	73	100	.08
Large granular lymphocytosis (%)	13	83	.0004
Calcification in basal ganglia (%)	7	33	.10
Laboratory data			
IgG (mg/dL, mean ± SD)	2213 ± 1104	1682 ± 464	.11
IgE (IU/mL, mean ± SD)	282 ± 298	2774 ± 3774	.04
VCA IgG (geometric mean titer)	2405	446	.01
EA IgG (geometric mean titer)	831	119	.02
EBNA (geometric mean titer)	30	45	.24
Viral load			
PBMC (copies/μg DNA, mean ± SD)	$10^{4.1 \pm 0.5}$	$10^{4.4 \pm 0.4}$	.09
Plasma (copies/mL, mean ± SD)	$10^{2.9 \pm 1.1}$	$10^{2.4 \pm 2.1}$	.49

HMB indicates hypersensitivity to mosquito bites; VCA, viral capsid antigens; EA, early antigens; EBNA, EB nuclear antigens; PBMC, peripheral blood mononuclear cells. Fisher exact test was used to compare symptoms between groups. Student *t* test was used to compare the mean copy numbers of EBV-DNA or laboratory data. Bold letters indicate statistically significant results.

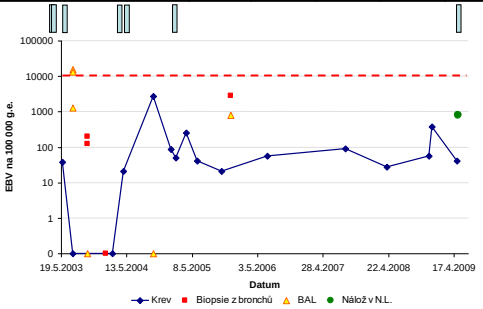
Table 2. Clinical features of 30 patients with chronic active Epstein-Barr virus infection

Symptoms and signs	(%)	Life-threatening complications	(%)
Fever	100	Hemophagocytic syndrome	21
Liver dysfunction	90	Coronary artery aneurysm	21
Splenomegaly	90	Hepatic failure	18
Lymphadenopathy	50	Malignant lymphoma	16
Thrombocytopenia	50	Interstitial pneumonia	12
Anemia	48	Central nervous system involvement	7
HMB	43	Sepsis	7
Skin rash	28	Pulmonary hypertention	4
Calcification in basal ganglia	18	Intestinal perforation	4
Oral ulcer	18	Myocarditis	4
Hydroa vacciniforme	14		

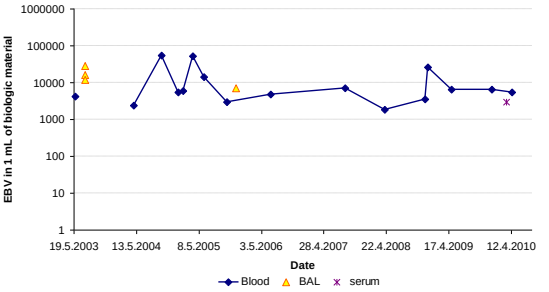
HMB indicates hypersensitivity to mosquito bites.

Kimura et al. Blood 15 July 2001, Vol. 98, No.2

Datum	6.2.2003	17.2.2003	27.5.2003	23.2.2004	26.3.2004	4.4.2005	4.5.2009
VCA IgG	+	+++		+	++	147 U/ml	119 U/ml
VCA IgM	+	+	-	+	+	72,5 U/ml	45,5 U/ml
EA-D	++	++	+++ (vysoká exprese)	++	++	90 U/ml	<150 U/ml
EBNA 1 IgG	-	-	-	+	+	52,5 U/ml	14,3 U/ml



EBV load in 1 ml of biological material



Malignant impact of EBV

NHL - Burkitt lymphoma

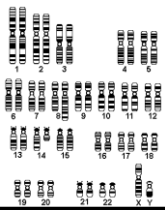
Very agressive

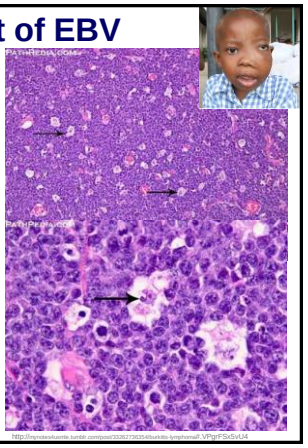
Picture of the **„Sky of stars "** – „stars" are apoptotic tumor cells which are fagocyte by macrophages; „sky" – represent tumor lymfocytes

Typical fusion t(8:14) chromosome 8 with c-myc oncogen

In the equatorial Africa incidence 5-15/100,000 of children

In Europe and USA 0,2-0,3/100,000 citizens





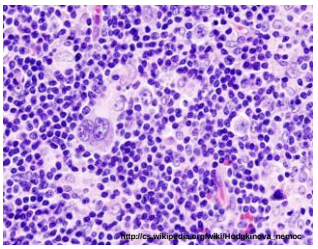
Malignant impact of EBV

Hodgkin lymfoma

High number of patients in long lasting remission.
Higher frequency in younger patients (approx. 20 yrs. of age) and in patients older 50-60 yrs. (median of age at dg. 35 yrs.)

Ratio of malignant and non-malignant cells approx. ~ 1:100

Incidence 2.4/100000 in ♀ and 3.1/100 000 ♂.

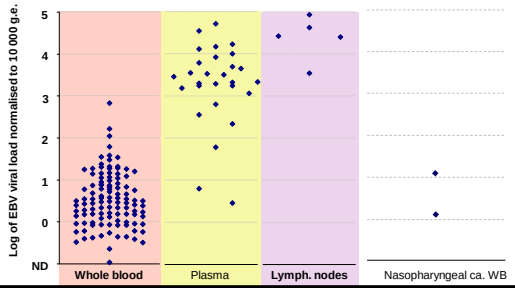


Histologically divided according to no. of **Reed-Sternberg's cells** (cells developed by mutation from B-cells) and according to the cellular frections:

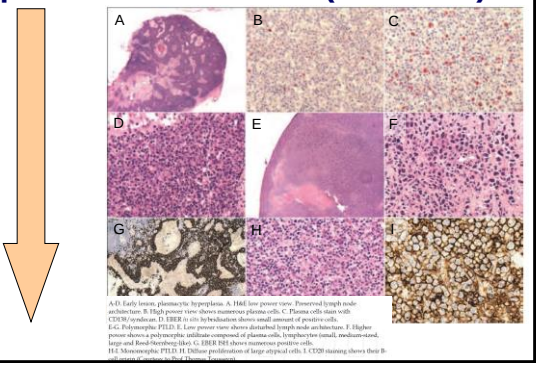
typ I with dominance of lymfocytes (few R-S cells, dominance of lymfocytes; best prognosis) (5 %);
typ II nodular-sclerotic (nodular centres, cells (reticular, lymphocytes, histiocytes) in colagen fibres) (70 %);
typ III mixed (20–25 %);
typ IV classical, few lymphocytes (No. of Sternberg's cells increased; worse prognosis) (1 %).

Patients with Hodgkin L. and NF ca.

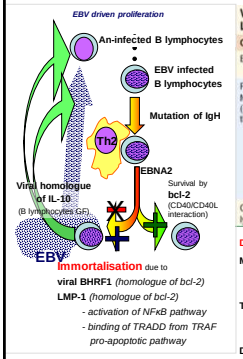
- Positive HL – 69 patients (38%)
  - positive 110 whole blood samples (17%) and 30 plasma samples (4.8%)
  - median of positivity in whole blood 3.45 copy (range 0.11 - 721)
  - median plasma positivity 5,400 copies/ml (range 600 – 126,600); after normalisation to 10 000 g.e median 2,500 (range 3 - 52 162)



EBV associated posttransplant proliferative disease (EBV-LPD)



Etiopathogenesis and classification EBV-LPD



Immortalisation due to
viral BHRF1 *(homologue of bcl-2)*
LMP-1 *(homologue of bcl-2)*
- *activation of NFκB pathway*
- *binding of TRADD from TRAF pro-apoptotic pathway*

World Health Organization Classification of Post-transplant Lymphoproliferative Disorder (PTLD)

Category	Subtype
Early lesions	Plasmacytic hyperplasia Infectious mononucleosis–like lesion
Polymorphic PTLD Monomorphic PTLD (classify according to lymphoma they resemble)	B-cell neoplasms – Diffuse large B-cell lymphoma – Burkitt lymphoma – Plasma cell myeloma – Plasmacytoma-like lesion – Other[a] T-cell neoplasms – Peripheral T-cell lymphoma NOS – Hepatosplenic T-cell lymphoma – Other
Classical Hodgkin lymphoma-type PTLD	cT1-2 gr 3 cT3-4

http://www.cancernetwork.com/oncology-journal/lymphoma-risk-and-response-after-solid-organ-transplant

Different symptoms of poly-, oligo- and monoclonal proliferation.

Mononucleosis-like syndrome

(fever, sore throat, myalgia, tonsillar hypertrophy and cervical lymphadenopathy, hepatopathy (bilirubinemia)

Tumorous form

(Symptoms secondary to the presence of lymphoid tumors: pain, obstruction, perforation, GI bleeding, respiratory distress, etc.)

Disseminated disease

(Proliferating B cells in blood and bone marrow, high fever and/or multi-organ failure)

EBV-LPD incidence and risk factors

Risk Factor	Degree of Risk	Study Reference(s)
EBV seronegativity pretransplant	24 × average risk	11–13
Younger age at transplantation	4–8 × adult risk	1,11
Type of immune suppression		
– Tacrolimus	2–5 × risk with cyclosporine	1,16,17
– OKT3 and/or ATG	3–4 × risk without these drugs	1
Type of organ transplant		9
Kidney	1%–3% of all transplant patients	
Liver	1%–3% of all transplant patients	
Hear	1%–6% of all transplant patients	
Heart-lung	2%–6% of all transplant patients	
Lung	4%–10% of all transplant patients	
Small bowel	20% of all transplant patients	
Time from transplant < 1 year	5–10 × risk at > 1 year	1
De novo CMV infection: CMV-positive recipient of a CMV-positive organ	4–6 × risk of CMV-negative recipient	21

ATG = anti-thymocyte globulin; CMV = cytomegalovirus; EBV = Epstein-Barr virus; OKT3 = muromonab-CD3 (Orthoclone OKT3); PTLD = post-transplant lymphoproliferative disorder.

In allogeneic HSCT incidence 2-25%.

- Cumulative intensity of immunosupresive treatment
- Use of anti-T lymfocytic antibodies in conditioning and/or posttransplant treatment
- T-cell depleted graft
- Intensive GvHD treatment
- Activation about 60 days after HSCT

EBV-LPD diagnosis

Diagnosis of neoplastic EBV-LPD should fulfill at least 2 of the following criteria:

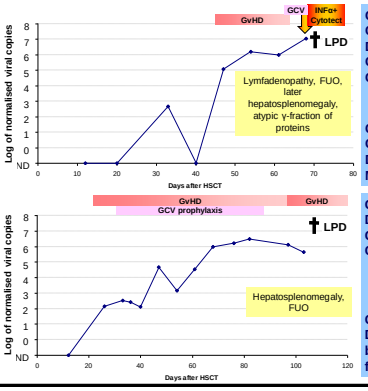
- **Change and/or destroy of the cell tissue culture by lymphoproliferative process**
- **Presence of monoclonal, or oligoclonal proliferation proven with cell and/or viral markers**
- **Evidence of EBV infection in many cells (e.g.. DNA, RNA, protein…**

EBV DNA detection in whole blood is not enough.

Die definice EBMT 1Dwp, 2007

- Clinical symptoms
- Imagine methods
- Immunology (Flow cytometry, Ig levels, clonality)
- Histology N.L. (detecting the presence of EBV)
- Direct detection of virus
  - EBV load (based mainly on NA detection)
    - Sample type: plasma, whole blood, MNC
    - Different methods of PCR – most frequently quantitative real-time PCR

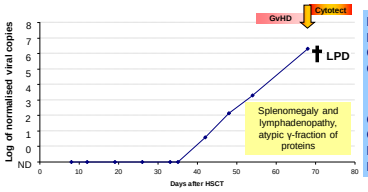
Retrospectively tested patients



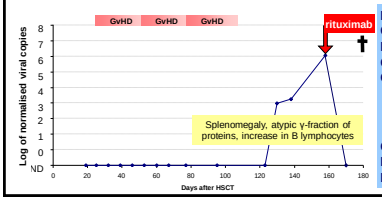
Girl, 5.5 yrs., HSCT for ALL CR2.
Donor: MMUD (8/10)
Graft: BM
Conditioning: TBI (12 Gy)
cyclophosphamide (120 mg/kg)
ATG (16 mg/kg)
GvHD prophylaxis: CyA+MTX
GvHD: grade III (D+ 49; GIT)
Deceased on D+74 due to MOF.

Girl, 4 yrs., HSCT for AML PR2
Donor: MMUD (9/10)
Graft: BM
Conditioning: busulphan(16 mg/kg)
cyclophosphamide (120 mg/kg)
melphalan (140 mg/m²)
ATG (40 mg/kg)
GvHD: grade III (D+ 25; GIT)
Deceased on D+117 due to bleeding and respiratory failure.

Retrospectively tested patients

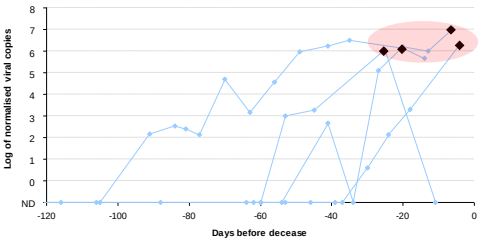


Boy, 5 yrs., HSCT for JMML
Donor: MUD
Graft: BM
Conditioning: busulphan(18 mg/kg)
cyclophosphamide (120 mg/kg)
ATG (16 mg/kg)
GvHD prophylaxis: CyA+MTX
GvHD: grade II (D+ 56; GIT)
Deceased on D+72 due to MOF.



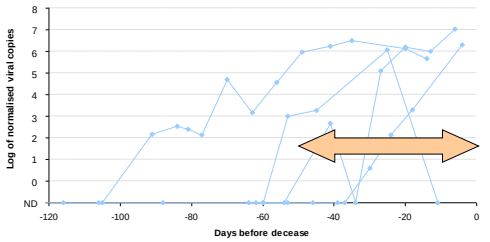
Boy, 11 yrs., HSCT for AML CR2.
Donor: MUD
Graft: BM
Conditioning: busulphan(16 mg/kg)
cyclophosphamide (120 mg/kg)
melphalan (140 mg/m²)
ATG (40 mg/kg)
GvHD: grade III-IV (D+ 23; GIT)
Deceased on D+182 due to MOF.

Retrospectively tested patients



Maximum detected quantity was between
$1.16x10^6$ and **$1.17x10^7$ NVCs**

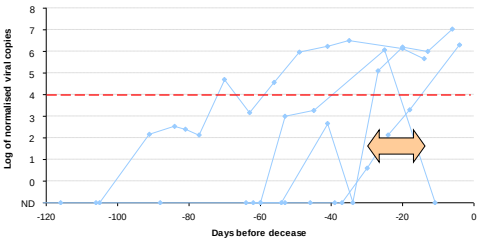
Retrospectively tested patients



Detection preceded decease with median of 47 days (-91 to -30)

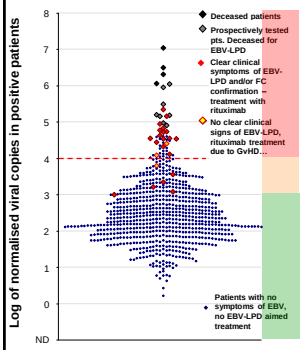
Detection preceded clinical signs of EBV-LPD with median of 35 days (-77 to -24)

Retrospectively tested patients



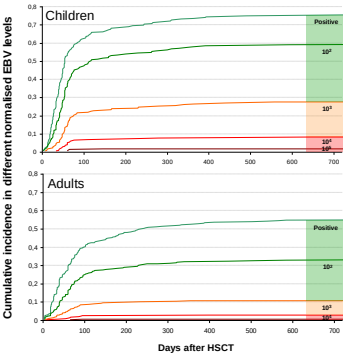
Quantity > 10^4 NVCs preceded clinical signs of EBV-LPD with median of 14 days (-56 to 2)

Prospective testing – maximal quantity



- **Very high risk level reactivation**
  > 10^4 NVCs – ≈ 50 IU/ng
  suspected and/or correlated with signs of EBV-LPD - 102 samples from 26 children (7.1%) and 22 adults (3.2%)
  EBV-LPD was confirmed in 22 (45%)
- **Risk level reactivation**
  > 10^3 and < 10^4 NVCs – ≈ 5-50 IU/ng
  687 samples from 95 (26%) children and 104 adults (15%)
- **Benign level reactivation**
  < 10^3 NVCs ≈ 5 < IU/ng
  low quantities with no clinical signs of EBV were detected in majority of samples from 275 children and 481 adults.

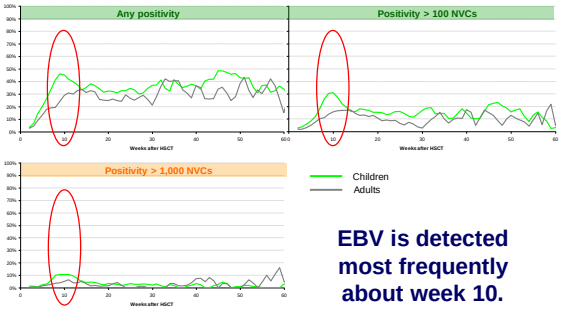
Prospective testing – incidence in time



Higher incidence in paediatric patients at every level

p<0.007

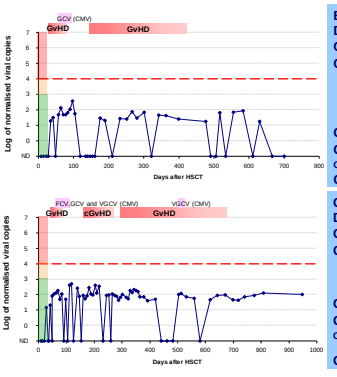
Proportion of positive patients by week and level



EBV is detected most frequently about week 10.

Benign level reactivation

< 10^3 NVCs ≈ 5 < IU/ng

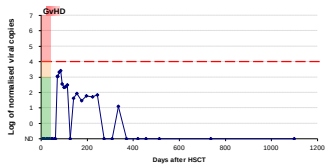


Boy, 13 yrs., HSCT for MDS-RAEB
Donor: MMUD (8/10)
Graft: PBSC
Conditioning: busulphan (16 mg/kg)
cyclophosphamide (120 mg/kg)
melphalan (140 mg/m²)
ATG (40 mg/kg)
GvHD prophylaxis: CyA+MTX
GvHD: grade II (D+28;GIT)
Other: BKV-HC(D+40), CMV(D+55)
Outcome: alive, no clin. problems

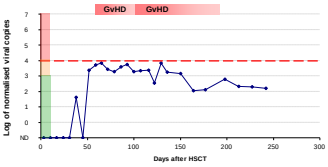
Girl, 13 yrs., HSCT for SAA
Donor: MMUD (9/10)
Graft: BM
Conditioning: TBI (5,4 Gy)
cyclophosphamide (200 mg/kg)
ATG (40 mg/kg)
GvHD prophylaxis: CyA+MTX
GvHD: grade II (D+40;GIT)
Other: CMV(D+46), lung affection of unknown ethiology, steroid DM
Outcome: alive, no clin. problems

Risk level reactivation

> 10^3 < 10^4 NVCs ≈ 5 - 50 IU/ng



Boy, 6.5 yrs., HSCT for ALL
Donor: RD (10/10)
Graft: BM
Conditioning: TBI (12 Gy)
etoposide (60 mg/kg)
ATG (40 mg/kg)
GvHD prophylaxis: CyA+MTX
GvHD: grade II (D+42;GIT+skin)
Outcome: alive, no clin. problems



Boy, 15 yrs., HSCT for BAL(ALL/AML)
Donor: MMUD (9/10)
Graft: PBSC
Conditioning: TBI (12 Gy)
etoposide (60 mg/kg)
ATG (40 mg/kg)
GvHD prophylaxis: CyA+MTX
GvHD: grade I (D+66)
Other: pulmonary mycosis
Outcome: alive, no clin. problems

EBV-LPD

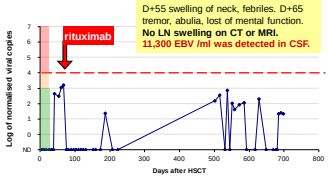
- **Detected: 28 patiens** (2.65% of tx, 3.3% of EBV positive)
  15 adults (1.98%) and 13 children (3.5%)
- **Mononucleosis like syndrom:** 1 adult
  peak at 54 days after HSCT (1,198 NVCs)
- **Localised :** 12 patients (9 adults, 3 children)
  median peak level at 68 days after HSCT
  median peak level 32,400 NVCs
- **Generalized:** 15 patients (10 children, 5 adults)
  median of peak level at 71 days after HSCT (range 41-230)
  median peak level 56,600 NVCs (27,407-220,716)

Confirmed by Flow cytometry.

Rituximab therapy was successful in all but 1 patient.

- **EBV 1 was detected in all but one patient with EBV 2.**

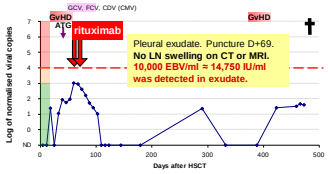
Localised EBV-LPD



D+55 swelling of neck, febriles. D+65 tremor, abulia, lost of mental function.
No LN swelling on CT or MRI.
11,300 EBV /ml was detected in CSF.

Woman,58 yrs.,HSCT for MDS/AML
Donor: SD (8/10)
Graft: PBSC
Conditioning: idarubicine (21 mg/m²)
fludarabine (25 mg/m²)
TBI (12 Gy) ATG (40 mg/kg)
GvHD prophylaxis: CyA, MMF
GvHD: grade II (D+22)

Outcome: rapid improvement,alive

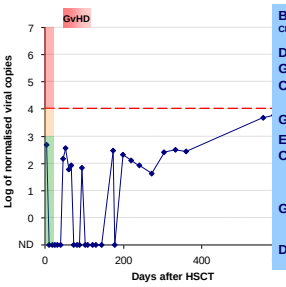


Pleural exudate. Puncture D+69.
No LN swelling on CT or MRI.
10,000 EBV/ml ≈ 14,750 IU/ml
was detected in exudate.

Woman, 39 yrs., HSCT for AML
Donor: MMUD (9/10)
Graft: PBSC
Conditioning: idarubicine (21 mg/m²)
fludarabine (25 mg/m²)
TBI (12 Gy) ATG (40 mg/kg)
GvHD prophylaxis: CyA+MMF
GvHD: grade III-IV (D+15)
Other: pulmonary proces of unknown origin
Outcome: deceased on D+478 due to MOF

Patient 3

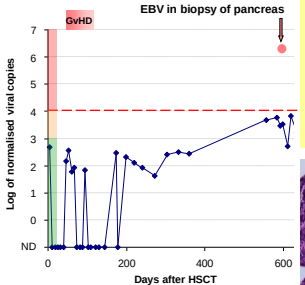
Localised EBV-LPD (NHL)



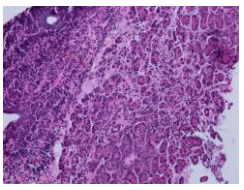
Boy, 15 yrs., HSCT for ALL in 1. CR
CMV, EBV, HSV seronegative
Donor: MSD (brother)
Graft: PBSC
Conditioning: TBI (12 Gy)
etoposid (60 mg/kg)
GvHD prophylaxis: CyA
Engraftment: D+14
Chimaerism: CC D+28
D+95 – 20% autologous (negative MRD)
D+130 – 1% autologous (negative MRD)
GvHD: grade II (D+28;GIT, skin)
Th: MP (1 mg/kg; until D+74) + CsA (until D+102)
D+280 – Herpes zoster (ACV)

7-12

Localised EBV-LPD (NHL)

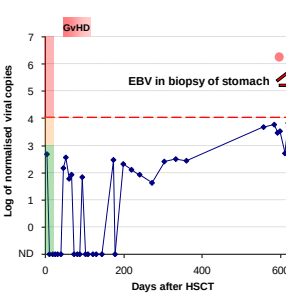


Pancreatopathy of unknown origin, confirmed by CT and US.
MRD negative.
↑CRP, ↑ IgG, no autoantibodies.
Laparotomy.
All types of lymphocytes detected in the biopsy. No malignant cells.
DR+ lymphocytes detected



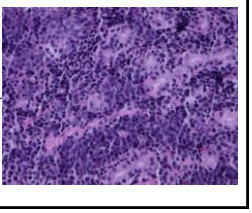
Patient 3

Localised EBV-LPD (NHL)



Anaemia. Gastric bleeding.
4 x rituximab 375 mg/m²

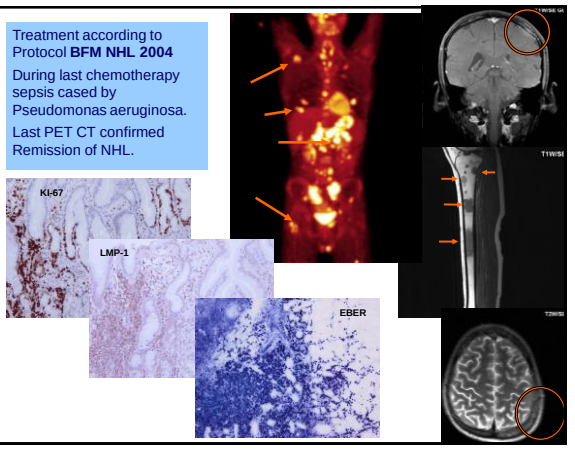
Gastric biopsy proves EBV+ Non Hodgkin Lymphoma (DLBCL)
Malignant cells of donor origin.



Treatment according to Protocol **BFM NHL 2004**

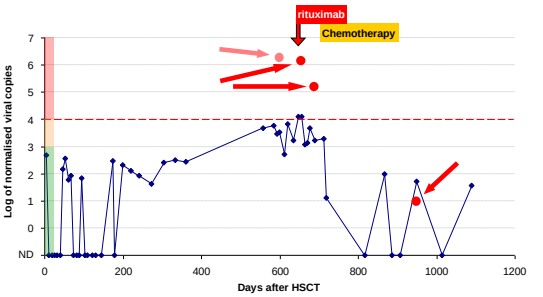
During last chemotherapy sepsis cased by Pseudomonas aeruginosa.

Last PET CT confirmed Remission of NHL.



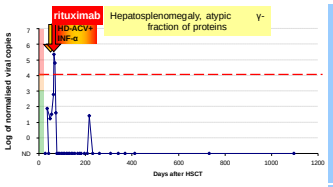
Patient 3

Localised EBV-LPD (NHL)

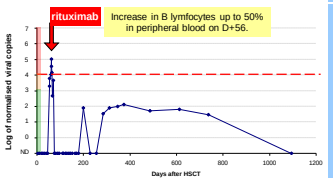


The patient remains in the remission of ALL and NHL.

Generalized EBV-LPD

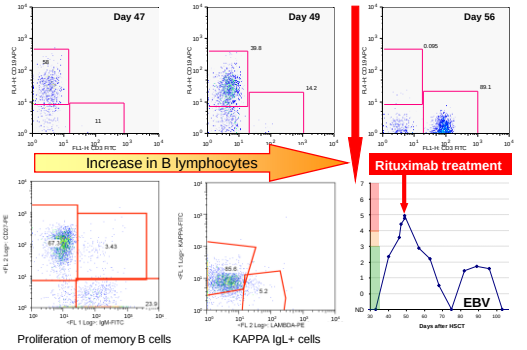


Boy, 3.3 yrs., HSCT for MPS type I
Donor: MMUD (5/6)
Graft: CB
Conditioning: busulphan (24.5 mg/kg)
cyclophosphamide (200 mg/kg)
ATG (40 mg/kg)
GvHD prophylaxis: CyA, MP
GvHD: grade II (D+49; GIT)
rituximab on D+69
Outcome: alive, no clin. problems



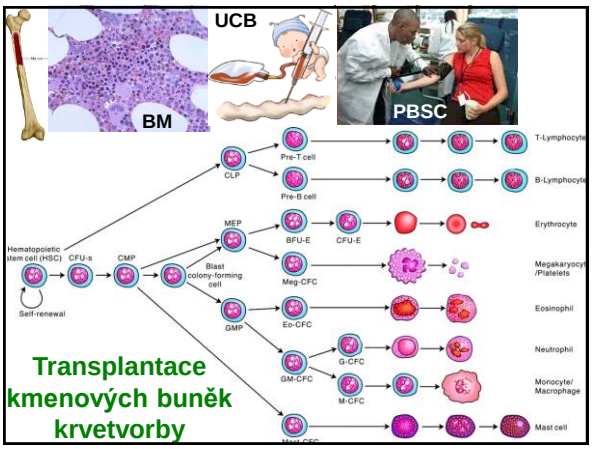
Boy, 4.4 yrs., HSCT for WAS
Donor: MUD
Graft: BM
Conditioning: busulfan (15.3 mg/kg)
cyclophosphamide (200 mg/kg)
ATG (40 mg/kg)
GvHD prophylaxis: CyA, MTX
GvHD: grade II (D+54; GIT)
rituximab on D+56.
Outcome: alive, no clin. problems

Flow cytometry EBV-LPD confirmation



Proliferation of memory B cells

KAPPA IgL+ cells



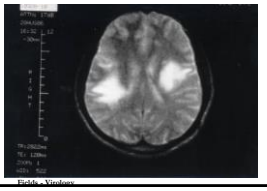
Transplantace kmenových buněk krvetvorby

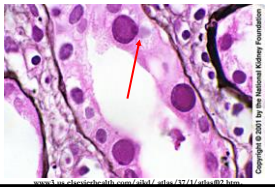
Polyomaviruses

- small ds DNA viruses with circular NA
- Capsid diameter 42-45 nm, genome: 5 kbp
- Transmission by fecal-oral route

- **JC virus** – progressive multifocal leukoencephalopaty PML
- **BK virus** – hemorrhagic cystitis, nephropathy (graft rejection in kidney transplant)
- **WUV and KIV** – respiratory infections
- **MCV** – Merkel cell carcinoma virus (rare skin carcinoma)
- **HPyV 7-12** (Human Polyomavirus) – mainly skin viruses

- Potencially treatable with **cidofovir**






Thank you for your attention
Petr.Hubacek@Lfmotol.cuni.cz