Serological Diagnosis of Epstein Barr Virus

L Ross Whybin
SEALS Serology

Epstein Barr Virus (EBV)

- Family Herpesviridiae, subfamily gammaherpesvirinae, genus lymphocryptovirus
- ds DNA enveloped virus
- Nucleocapsid 100-110nm in diam; with 162 capsomers
- Aymmetrical material surrounding capsid designated the tegument (structures between the capsid & envelope)
- Envelope containing viral glycoprotein spikes on its surface
- Membrane is derived by budding of immature particles through cell membrane and is required for infectivity
- Genome is linear ds DNA molecule with 172 kbp
- Viral genome does not normally integrate into cellular DNA but forms circular episomes which reside in the nucleus
- Genome is large enough to code for 100-200 proteins but only a few have been indentified

Epstein Barr Virus (EBV)

- Family Herpesviridiae
- ds DNA enveloped virus
- Genome is linear with 172 kbp
- Genome is large enough to code for 100-200 proteins but only a few have been identified

Epstein Barr Virus (EBV)

2 peaks of infection: young pre-school(1-6) and adolescents/young adults (14-20)
Estimated 80-90% of adults are seropositive for EBV

Infectious mononucleosis (IM)
Chronic active EBV
Burkitt's Lymphoma
Nasopharyngeal Carcinoma
Lymphoproliferative disorders (immunocompromised)
X-linked lymphoproliferative syndrome
Oral hairy leukoplakia, diffuse polyclonal lymphomas, chronic interstitial pneumonitis in AIDS patients

Epstein Barr Virus (EBV)

- Symptoms:
- Sore throat (80-90%)
- Lymphadenopathy (cervical) present in majority of cases and may last several weeks
- Splenomegaly (50-60%)
- Hepatomegaly (15-25%)
- Jaundice (5-10%)
- Pharyngitis & palatal petechiae (grey-white membrane) first week
- Fever first 2 weeks
- Immunocompromised: GI symptoms, renal graft rejection/failure, lymphoproliferative disease, lymphoma

History of Serological testing for EBV

1932 Paul & Bunnell (sheep RBC's)
1975 Monospot (horse RBC's)
1966 EBV IgG & IgM IFA; cultivation of EBV infected lymphoid cell lines
1985 EBV EIA; EBV antigens from infected cells purified under solid-phase absorption
1986 EBV EIA; polypeptides with immunodominant epitopes prepared by recombinant technology

Testing for Heterophile Antibodies

Paul Bunnell + Monospot
IgM class, not EBV specific
90-98% sensitive in adults
Negative early in infection
May remain positive for up to 6-12 months
10-20% adults and up to 50% young children never develop heterophile Abs (false negative)
3-7% false positive rate due to long-term persistence of Ab
2-3% false positive results in patients with autoimmune diseases
Detected in other mononucleosis illnesses (primary CMV, Hep A, HIV, lymphoma)
Heterophile Ab detection + atypical lymphocytes support lab diagnosis of EBV

EBV Viral capsid antigen

Synthesized late in the lytic cycle

A complex of at least 7 structural proteins and glycoproteins making up the viral capsid.
Incl.
gp125 - major capsid protein
p18 - minor tegument protein

EBV VCA IgG ANTIBODY

- Appears early in primary EBV infection
- 4-7 days after symptoms
- May precede EBV VCA IgM: uncertainty in diagnosis from a single sample
- Usually persists for life
- EBV VCA IgG: acute, convalescent or past phase of infection
- High levels in Burkitt's lymphoma and NPC

EBV VCA IgM ANTIBODY

- Indicator of recent primary EBV infection
- EBV VCA IgM usually present from 2-4 months after primary EBV infection
- Can be delayed, even absent in a small number of primary EBV infection in adults
- May persist for several months (10% for 6-8 months) after infection
- May re-appear in reactivation of EBV
- Cross-reactivity with other Herpesviruses (VZV, HSV)
- False-positive in other acute viral infections (HIV, Parvovirus B19) and patients with IgM RF.
- False-negative with excess IgG/co-specific IgG blocking attachment sites
- Reactivation in Hepatitis A infection

Epstein Barr Virus Nuclear Antigen (EBNA)

Complex of at least 6 proteins (EBNA –1, -2, -3A, -3B, -3C & -LP)

EBNA-1 thought to be essential for maintenance of episomal state of EBV in infected cells and binds to the origin of replication

EBNA-1 expressed in all known virus carrying cells; expression may be lost when lytic cycle ensues

EBV EBNA-1 IgG ANTIBODY

Late (latent phase protein) marker of primary EBV infection, although may be present soon after onset of IM

Appears 3-6 months following infection; marks transition from acute to convalescence; indicates past or resolving infection

Peaks 3-12 months post infection, declines but remains detectable indefinitely

Up to 6% of infections never develop EBNA-1 IgG antibody (higher in immunocompromised) (Bauer 1994)

In severely immunocompromised patients, EBNA-1 IgG may decline to low or undetectable levels in response to increase in productive EBV replication

EBV EBNA-2 IgG ANTIBODY

- EBNA-2 IgG antibodies appear early in EBV infection
- May be present in up to 30% of individuals at time of onset of disease
- Presence of EBNA-1 IgG and absence of EBNA-2 IgG excludes primary infection
- Ratio of EBNA-1 Abs vs EBNA-2 Abs used for the serodiagnosis of EBV reactivation
- No commercial assays for EBNA-2 Ab available

EBV EBNA IgM ANTIBODY

- Indicator of recent primary EBV infection
- EBNA IgM usually present from 2-4 months after primary EBV infection
- May persist for several after infection
- May re-appear in reactivation of EBV
- Cross-reactivity with other Herpesviruses (VZV, HSV)
- False-positive in other acute viral infections (HIV, Parvovirus B19) and patients with IgM RF.
- False-negative with excess IgG/co-specific IgG blocking attachment sites

EBV EA (Early Antigen) ANTIBODY

- EA is a complex of proteins only expressed in infected cells undergoing lytic cycle
- Early antigen/diffuse (EA/D) & Early antigen/restricted (EA/R)
- EA/D Abs rise during acute infection and fall to undetectable levels within 3-6 months
- EA/R remain elevated for up to 2 years
- 30-70% of patients with acute EBV develop EA/R and EA/D Abs
- High levels of EA/R detected in Burkitt's lymphoma
- High levels of EA/D IgG and EA/D IgA in NPC

EBV VCA IgA & EA/D IgA Abs

- Induced during acute primary EBV
- Persistent high levels in NPC
- Negative predictive value/Sensitivity approx. 97%
- Positive predictive value 0.5-2% VCA IgA in NPC (high risk populations)
- Positive predictive value VCA IgA + EA/D IgA in NPC rises to 20%
- Used for screening in very selected groups (middle aged to elderly Southern Chinese with family history of NPC)
- Rising titres indicate progression or relapse

EBV VCA IgG Avidity Index

- Can distinguish recent from past or reactivated infection particularly where VCA IgM persists long-term
- B cells switch from IgM to IgG isotype in vivo; the first IgG Abs produced are of low avidity
- Later IgG Abs mature through somatic hypermutation in the IgG DNA-encoded region and B cell clones end up producing relative higher avidities
- AI: ratio between urea-treated and non-urea treated sample
- Improved sensitivity for diagnosis from 93% to 100%
- AI: 54% at 6 weeks
- AI: 82% at 28 weeks

EBV Western Blot

- Classical lysate blot assays with EBV transformed cells
- Line blot assays with recombinant antigens incl.
  p72 (EBNA-1)
  p18 (VCA)
  p23 (VCA)
  p54 (EA)
  p138 (EA)
- Detects EBV specific antibodies to multiple EBV-specific antigens simultaneously
- Useful confirmatory method

OTHER EBV SEROLOGY

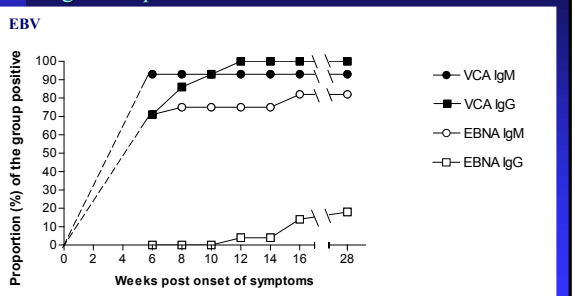
- EBV VCA IgA: BL, NPC
- EBV EA (D/R) IgG, IgA: BL,NPC
- EBV Western Blot: Confirmatory method
- EBNA-2 IgG: Primary infection, Reactivation

EBV serology

- Dubbo study: 28 well characterised EBV cases
- Specificity: 30 cases previous EBV & recent HIV, CMV or Hepatitis A (FP EBV serology)

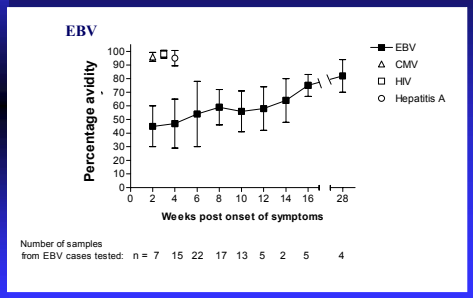
The Dubbo Cohort Study

Serological responses



The Dubbo Cohort Study

Serological responses (IgG VCA avidity)



The Dubbo Cohort Study

Serological responses (IgG VCA avidity)

	Sample size (n)	Number (%) of group with positive serology VCA IgG	VCA IgM	EBNA IgG	EBNA IgM	IgG VCA avidity (mean % and SD)
Hep A	15	15 (100)	12 (80)	15 (100)	12 (80)	95 (5.7)
HIV	14	14 (100)	5 (36)	14 (100)	10 (71)	98 (2.8)
CMV	6	6 (100)	1 (16)	6 (100)	6 (100)	96 (3.2)
Combined	35	35 (100)	18 (51)	35 (100)	28 (80)	96.3 (4.4)

VCA IgG (gp125) & EBNA-1 IgG

Age 2 - 50 yrs (n=3317)

		VCA IgG +	VCA IgG -
EBNA IgG	+	*59%*	*6%*
	-	*11%*	*24%*

VCA IgG(gp125) & EBNA-1 IgG

Age 10 - 20yrs (n=552)

		VCA IgG +	VCA IgG -
EBNA	+	53%	6%
IgG	-	15%	26%

VCA IgG (gp125) & EBNA-1 IgG

Age > 50 yrs (n=747)

		VCA IgG[1] +	VCA IgG[1] -
EBNA	+	76%	11%
IgG	-	8%	5%

EBV VCA p18 IgG ANTIBODY

- Highly immunogenic in humans
- Recognised by healthy EBV-seropositive persons worldwide
- Found in 'most' EBV carriers-(Wout 1993)
- A late marker of EBV infection.(Hinderer 1999)
- Not lost during immunosuppression (Bauer 2001)
- Does not appear to have sequence homologues to other human herpesviruses

Previous EBV Infection

53 Samples

VCA gp125 IgG Negative / EBNA-1 IgG Positive

gp125 IgG	p18 IgG	No
Neg	**Pos**	**52**
Neg	**Neg**	**1**

Anti EBV VCA p18 in recent infection

VCA IgM pos / EBNA IgG neg (n=32)

12/32 anti p18 neg.

VCA IgM pos / EBNA IgG neg/Avidity<60%. (n=12)

5/12 anti p18 neg.

EBV VCA p18 IgG ANTIBODY CONCLUSIONS

- EBV VCA p18 IgG EIA appears more sensitive than EBV VCA gp125 IgG EIA
  (except early acute EBV)
- EBV VCA p18 IgG EIA agrees better than EBV VCA gp125 IgG EIA with EBNA IgG EIA (52/53)
- EBV VCA p18 useful to assist in the determination of EBV immune status

EBV summary

VCA-IgM antibody appears in both primary infection & reactivation of EBV.

VCA IgG antibody appears early in primary infection and should last for life.

Low avidity IgG antibody only appears in primary EBV infection & increases to approx 80% by 6 months.

EBNA IgG antibody appears after about 3 months & should last for life.

The combination of low VCA IgG avidity with positive VCA IgM & negative EBNA IgG is 100% specific for the diagnosis of primary EBV infection.

RCPA QAP L1:2005:1A,1B

TESTS	PARTICIPANTS	MANUFACTURERS
EBV VCA IgM EIA	70	12
EBV VCA IgG EIA	62	14*
Heterophile Screen	40	12
EBNA IgG EIA	25	7
EBV EA IgG EIA	2	2
EBV EA & EBNA IgA EIA	2	2
EBNA IgM EIA	1	1
EBV Avidity Index	1	1

RCPA QAP L1:2005:1A,1B

- EBV VCA IgG EIA: 62 participants
- PanBio EBV VCA gp125 IgG: 16/62
- PanBio EBV VCA p18 IgG: 11/62
- Trinity Biotech EBV VCA p18 IgG: 9/62

EBV Serology

- EBV VCA IgG: Negative
- EBV VCA IgM: Negative
- EBNA IgG: Negative
- EBV AI: N/A

No evidence of past infection.
If early in course of illness, repeat blood.
Suggest EBV VCA p18 IgG.

EBV Serology

- EBV VCA IgG: Negative
- EBV VCA IgM: Positive
- EBNA IgG: Negative
- EBV AI: N/A

Early EBV or False Positive
Suggest EBV VCA p18 IgG Ab
Suggest repeat blood

EBV Serology

- EBV VCA IgG: Positive
- EBV VCA IgM: Negative
- EBNA IgG: Negative
- EBV AI:<60%

Probable acute EBV, suggest repeat

EBV Serology

- EBV VCA IgG: Positive
- EBV VCA IgM: Negative
- EBNA IgG: Negative
- EBV AI:>60%

Probable past infection

EBV Serology

- EBV VCA IgG: Positive
- EBV VCA IgM: Negative
- EBNA IgG: Positive
- EBV AI: >60%

Past EBV

EBV Serology

- EBV VCA IgG: Negative
- EBV VCA IgM: Negative
- EBNA IgG: Positive
- EBV AI: N/A

Probable past infection

EBV Serology

- EBV VCA IgG: Positive
- EBV VCA IgM: Positive
- EBNA IgG: Positive
- EBV AI: >60%

Past infection (>=3 months)

EBV Serology

- EBV VCA IgG: Positive
- EBV VCA IgM: Positive
- EBNA IgG: Negative
- EBV AI: <60%

Acute EBV