American Association of Bioanalysts

AAB 2013 Annual Meeting & Educational Conference

Occult Hepatitis and HIV Prophylaxis

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Hepatitis C Virus Infections

- HCV is a leading cause of chronic liver disease worldwide
- HCV infections
  - Acute
  - Chronic
  - Occult

Hepatitis C Virus Infections-Cont'd

- Diagnosis (Dx) for classic (acute & chronic) HCV infections is based upon the most sensitive standard clinical assays.
  - Antibodies against HCV (anti-HCV^{+}) &/or
  - HCV RNA^{+} in serum ($sRNA^{+}$)
- Chronic HCV is defined as serum viral RNA^{+} for more than 6 months.

Occult Hepatitis C (OHC) Virus Infections

- 2004 - “occult” HCV infections described (absence of conventional viral markers)
  - Anti-HCV^{-}
  - sRNA^{-}, but
  - Elevated liver enzymes ($_{l}$enzymes↑), and OHC patients will have:
    - ~57% liver biopsies positive for viral RNA ($_{l}$RNA^{+}), and
    - ~70% have peripheral blood mononuclear cells (PBMCs) positive for viral RNA (pbmc-RNA^{+})

V. Carreno, J. Bartome *et al*., World J. Gastro. **18(23):** 2887-2894, 2012.

Occult Hepatitis C (OHC) Virus Infections – Cont'd

- Other clinically occult HCV infections
  - Anti-HCV^{+}
  - sRNA^{-}, but 90% are $_{l}$RNA^{+}, and pbmc-RNA^{+}, and
  - Have normal liver enzymes ($_{l}$enzymes→)
- Occult HCV infections have been reported in:
  - Hemodialysis patients
  - Families with patients having OHC virus infections
  - Healthy subjects with normal liver enzymes

Occult Hepatitis C (OHC) Virus Infections – Cont'd

- **OHC virus infection outcomes/issues**
  - Induces liver damage; regression analysis of liver histology data indicates necro-inflammatory activity & liver fibrosis independently associated with OHC virus infections[1]
  - Results in viral release into the blood
  - Intrafamilial transmission of HCV after contact with OHC virus infected family members[2]

[1] I. Castillo, M. Pardo *et al*., J. Infect. Dis. **189:**7-14,2004.
[2] V. Carreno, J. Bartolome *et al*., Rev. Med. Virol. **18:**139-157,2008.

Occult Hepatitis C (OHC) Virus Infections – Cont'd

- **OHC virus infection outcomes/issues – cont'd**
  - Associated with liver cancer
  - Evidence suggests that OHC viral infections may have a role for liver failure in liver transplanted patients
  - Virus persistent for years
  - Persistent virus can be reactivated; a patient with "cleared" chronic HCV became $sRNA^-$ for 8.5 years, had normal liver enzymes and had HCV re-emerge ($sRNA^+$) following prednisone therapy[1]

[1] W.M. Lee, J.E. Polson, *et al*., J Infect Dis **192**:1088-1092, 2005.

Occult Hepatitis C (OHC) Virus Infections – Cont'd

- **OHC virus reactivation** (re-appearance of serum viral RNA) – anti-HCV positive patients with occult HCV infections have recurrence of serum viral RNA if:
  - On long-term chemotherapy
  - Receiving immunosuppressive therapy for transplants
  - Immunocompromised

Occult Hepatitis C (OHC) Virus Infections – Cont'd

- **OHC Viral Diagnosis (Dx)**
  - Serum HCV-RNA (ultracentrifugation + RT-PCR)
  - HCV-RNA in PBMC (RT-PCR)
  - Genomic and anti-genomic viral strand-specific RT-PCR
  - Anti-HCV (non-commercial ELISA)
  - Anti-GOR (ELISA); GOR47-1 is a host-cell gene product which cross reacts with HCV core protein sequence[1]

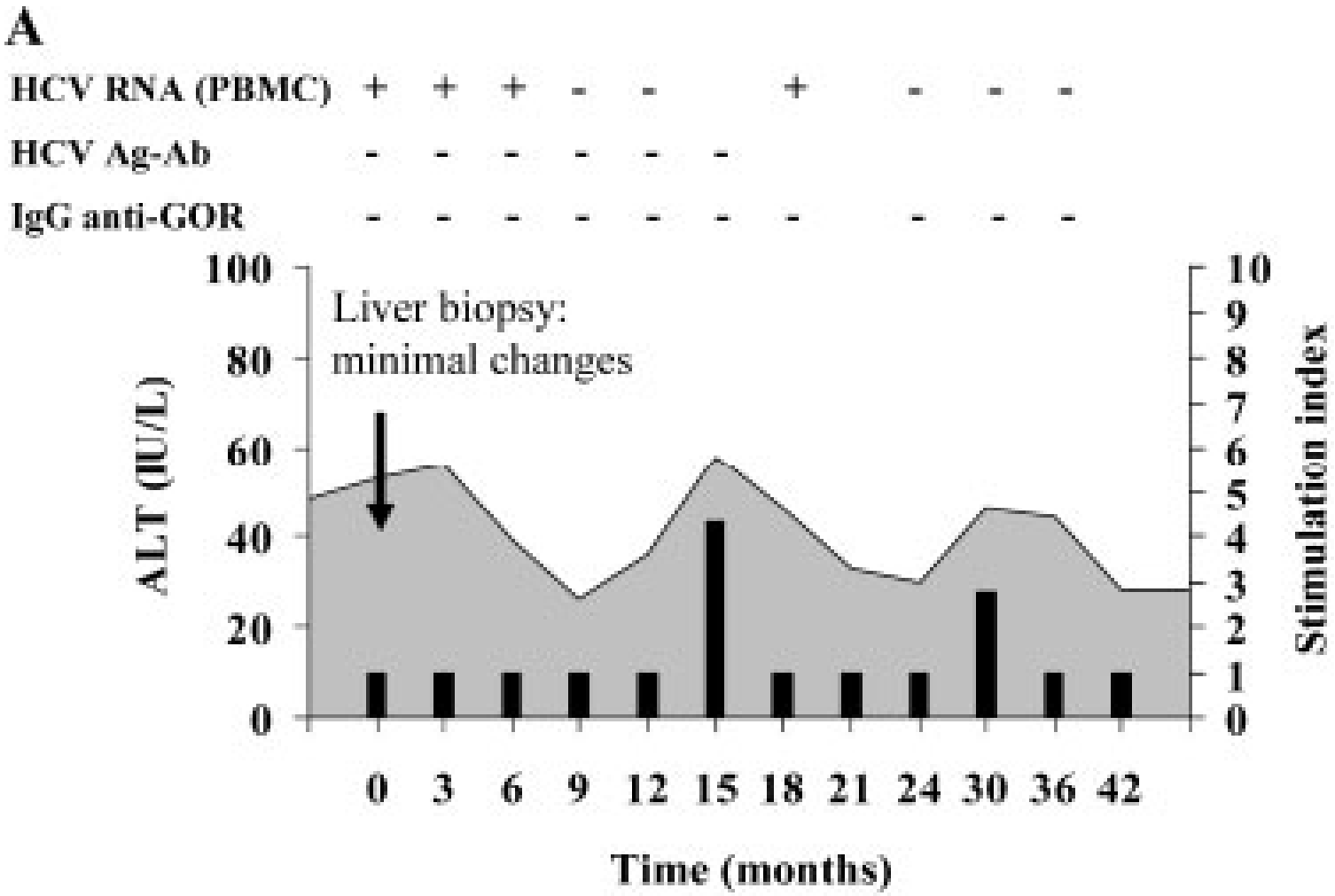
[1] JA Quiroga, I. Castillo, *et al*., Clin Vaccine Immunol, **14**:1302-1306, 2007.

Occult Hepatitis C (OHC) Virus Infections – Cont'd

- **OHC Viral Diagnosis (Dx)-cont'd**
  - T-cell HCV-specific responses
  - Serial testing is essential for negative patients. Negative patients should be serially tested in both compartments (serum & PBMCs) every 3-4 months for one year before excluding an occult HCV infection[1]

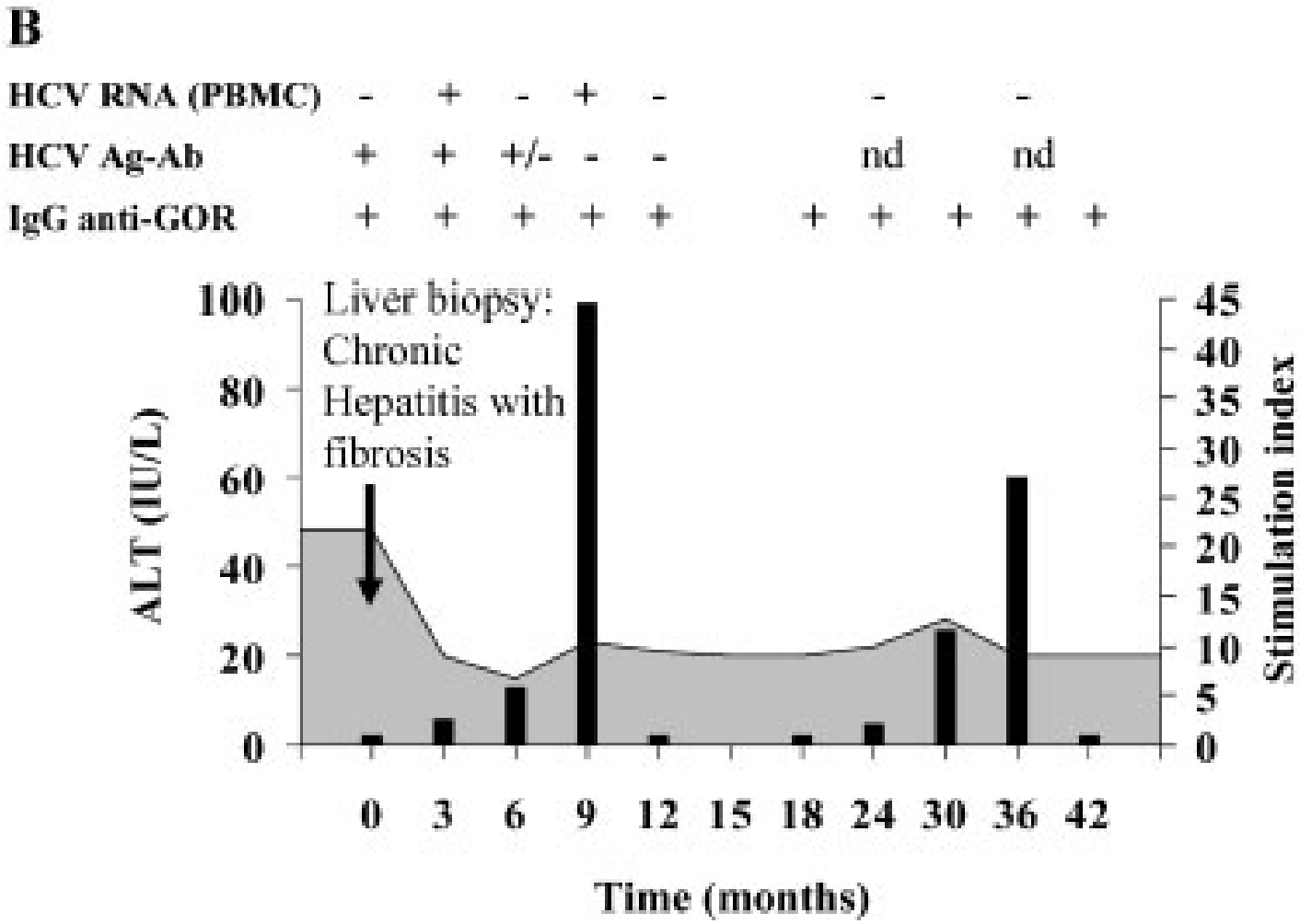
[1] V. Carreno, J. Bartolome *et al*., Rev. Med. Virol. **18:**139-157,2008.

Serologically Silent OHC Virus Infection



Copied From & Cited in V. Carreno, J. Bartolome *et al*., Rev. Med. Virol. **18:**139-157,2008.

Serologically Silent OHC Virus Infection-cont'd



Copied From & Cited in V. Carreno, J. Bartolome *et al*., Rev. Med. Virol. **18:**139-157,2008.

Occult Hepatitis C (OHC) Virus Infections – Cont'd

- **Humoral immunity -** *for some patients*
  - anti-HCV antibodies:
    - Develop late
    - Do not develop at all
    - Are generally low-titered
    - Can rapidly decrease
    - Decrease upon follow-up
    - Disappear for non-structural proteins 4 and 5
  - Thus, humoral immunity often is limited

Occult Hepatitis C (OHC) Virus Infections – Cont'd

- **OHC virus immunology**
  - 40% of OHC cohorts that were anti-HCV^{-} by a commercial screening test were anti-HCV^{+} by a non-commercial conserved virus core peptide sequence
  - Non-reactive specimens for anti-HCV core antibodies may become positive upon serial testing
  - Anti-HCV core was detected in 23% of family members/relatives of anti-HCV^{-} patients

Occult Hepatitis C (OHC) Virus Infections – Cont'd

- Dx – most OHC virus infections can be diagnosed without a liver biopsy
- 91% of patients with OHC viral infections could be diagnosed by anti-HCV core antigen testing, ultra-fuged serum testing for viral RNA (RT-PCR), and pbmcs-RNA testing (viral RNA RT-PCR)

Summary – Occult Hepatitis C (OHC) Viral Infections

- OHC defined as elevated or non-elevated liver enzymes, anti-HCV$^{-/+}$ and serum viral RNA^{-}
- Not detected by current, conventional commercial testing (as of 2013)
- Diagnosis is enhanced by serial sampling : 1) of “fuged” serum; 2) of PBMCs for viral RNA and 3) for unique, specific anti-HCL responses
- Surrogate (GOR) marker detection can be helpful as well as HCV-specific T-cell responses

Summary – Occult Hepatitis C (OHC) Viral Infections (Cont'd)

- OHC viral infections appear to be milder than chronic HCV viral infections
- OHC viral infections can persist for many years
- Persistent OHC infections can be re-activated
- Long-term outcomes of OHC virus infections are unknown

Therapy & Prophylaxis For HIV Sanctuary Sites

- Generally, therapy does not cure (exception!)
- Antiretroviral therapy (ART) often does not eliminate HIV cellular reservoirs
- Prophylaxis has limitations

Therapy & Prophylaxis For HIV Sanctuary Sites-Mammary Glands

- Increased risk of HIV transmission via breast feeding.
  - Mothers that acquires HIV during lactation
  - Later stages of maternal HIV infections
  - Length & intensity of breast feeding
  - Mammary gland Inflammation
- In mammary tissue, cell associated virus-levels are barely affected by maternal ART.

P. Van de Perre, Pierre-Alain Rubbo *et al.* www.ScienceTranslationalMedicine.org pp1-14, 18 July 2012 **4(Issue 143)** 143s3.

HIV Prophylaxis & Breastfeeding

Conditions	HIV Transmission Rate @ 6 Weeks Post Birth	HIV Transmission Rate @ 6 Months Post Birth
Without ART & Prophylaxis	14.1 %	19.5 %
With Triple-ART Pre & Post-Birth	3.3 to 4.2%	1.1 to 8%

Cited From: J. Ndirangu, J. Viljoen *et al*. PLOS ONE,**7(12):**1-8, Dec. 28,2012, e51493. doi:10.1371/journal.pone.00511493.

Prevention/Prophylaxis of Sexually Transmitted Pathogens (HIV)

- Condoms
- Male circumcision
- Treatment of infected partners
- Risk-reduction counseling
- Voluntary testing
- Abstinence
- Sero-sorting
- **Pre-exposure Prophylaxis (PrEP), Post-exposure Prophylaxis (PEP) & PrEP + PEP**

Strategies for Reducing Sexual HIV Transmission

Method	% Reduction	Comments	Source
Condoms	~80	Meta-analysis	Weller *et al*., Cochrane Report, 2002.
Circumcision	~65	Benefits males	Wawer, *et al*., Lancet, 2009
Pre –exposure prophylaxis (topical)	~39-54 with tenofovir	Heterosexual ,S. African Study	Abdool-Karim, *et al.,* Science, **329:**1168-74, 2010.
Pre –exposure prophylaxis (oral)	~44-73 with Truvada	International MSM Study	Grant, *et al*., NEJM, **363:**2587-99, 2010.
Post –exposure prophylaxis (oral); also pre + post			

Adapted & Modified From: C. De Mendoza, AIDS Rev., **13:**57-59, 2011.

Possible Reasons for HIV Pre-Exposure Prophylaxis Trial Failures

- Poor treatment compliance
- Engagement in riskier behaviors
- Increased levels of genital tract inflammation
- Insufficient genital tract drug concentrations
- Daily treatment alterations of mucosal immunity

Pre-Exposure Prophylaxis (PrEP) Issues

- How will compliance be monitored? compliance (< 10% for MSM study & 15% for heterosexual partners study)
- Self-reported pill usage & pill counts can be unreliable measures of compliance
- Objective measures of compliance are important
- Side effects (Truvada – irreversible renal toxicity, liver toxicity, significant decreases in hip and spine bone density)

Pre-exposure Prophylaxis (PrEP) for HIV-1

- Emitricitabine (Emtriva, FTC, an NRTI)
- Tenofovir (Viread)
- Tenofovir disoproxil fumarate (TDF, prodrug of tenofovir, an NRTI)
- TDF and FTC (Truvada), approved July 16, 2012 by FDA for prophylactic use (PrEP) against HIV

Synopsis of Interim Assessments for HIV PrEP in Heterosexual Adults

- Before initiating PrEP:
  - Document HIV^- antibody results
  - Test for HIV if symptomatic
  - Test for HIV if unprotected sex with HIV^+ person during last month
  - Determine if pregnant, planning to get pregnant, or breast-feeding (If yes, counsel)
  - Confirm if at very high risk for HIV infections

Adapted and modified from: MMWR **61(31)**:586-589, Aug. 10, 2012.

Synopsis of Interim Assessments for HIV PrEP in Heterosexual Adults (Cont'd)

- Determine if partner is HIV^+, on therapy and linked to healthcare
- Confirm kidney functions
- Screen for *HBV* infections
- Screen and treat as needed for STIs

- Initiating and continuing PrEP
  - Prescribe one Truvada pill daily
  - Generally prescribe for only 90 days

Synopsis of Interim Assessments for HIV PrEP in Heterosexual Adults (Cont'd)

  - Renew only if HIV testing is negative, pregnancy test is negative (informed of risks)
  - Provide risk reduction and medication adherence counseling
- Follow-up while on PrEP medication
  - Perform HIV antibody testing or 4th generation Ab/Ag testing every 2-3 months
  - Conduct pregnancy testing
  - Evaluate and support PrEP medication adherence

Synopsis of Interim Assessments for HIV PrEP in Heterosexual Adults (Cont'd)

- Evaluate more often if not compliant to adherence
- Assess risk behaviors and risk-reduction every 2-3 months
- Assess for STIs every 2-3 months, test and treat
- Test for bacterial STIs every 6 months
- Check kidney function after 3 months and then every 6 months

Pre-Exposure Prophylaxis (PrEP) Issues

- Still critical to have HIV testing (to prevent resistance & transmission of resistant variants).
- Still critical to have treatments for STIs.
- Effects on human fetus during 1st trimester are a concern.
- Expensive (cost to prevent 1 new infection, who pays and not affordable in all countries)

Pre-Exposure Prophylaxis (PrEP) Issues (Cont'd)

- Should major interventions be dealing more with upstream problems (behaviors) & less concern with prophylaxis.
- Key HIV epidemic drivers are most likely high rates of multiple concurrent & serial partners.
- Ethics – providing antivirals to healthy HIV^- subjects when HIV^+ individuals receive no treatment.

Informal HIV Pre-exposure Prophylaxis (PrEP) With Australian Homosexual Men

Associated Factors	N	% Using PrEP Before Non protected Anal Sex	Adjusted Odds Ratio (95% CI)
Residents-1	151	**0.7**	0.5
Residents-5	742	**4.3**	**2.48 (1.4 to 4.2)**
Age: 30-39 y	1065	**1.8**	0.5 (0.2 to 1.2)
Age:<25 y	669	**4.2**	1
Edu.: University	1963	**1.9**	0.7 (0.3 to 1.6)
Edu.: ≤3 y	257	**5.5**	1

Modified & Adapted From: I. B. Zablotska, G. Prestage *et al*., JAIDS **62(3):**334-338,2013.

Informal HIV Pre-exposure Prophylaxis (PrEP) With Australian Homosexual Men-Cont'd

Associated Factors	N	% Using PrEP Before Non protected Anal Sex	Adjusted Odds Ratio (95% CI)
Ethnic Group-1	2502	**1.9**	1
Ethnic Group-4	138	**7.3**	3.2 (1.4 to 7.0)
No. Partners-1	1068	**0.8**	1
No. Partners-≥11	592	**5.1**	2.2 (1.9 to 5.6)
Protected sex	1438	**1.5**	1
Non protected	479	**5.2**	2.7 (1.4 to 5.0)

Modified & Adapted From: I. B. Zablotska, G. Prestage *et al*., JAIDS **62(3):**334-338,2013.

Informal HIV Pre-exposure Prophylaxis (PrEP) With Australian Homosexual Men-Cont'd

Associated Factors	N	% Using PrEP Before Non protected Anal Sex	Adjusted Odds Ratio (95% CI)
Injected Drug use-Never	3517	**2.1**	1
Injected Drug use-Monthly	56	**19.6**	**2.6 (1.03 to 6.4)**
Party Drugs & Sex-Never (N)	2917	**1.8**	
Party Drugs & Sex-Monthly (M)	181	**8.8**	**P<0.001**
Party Drugs,& Group Sex- (N)	3193	**1.7**	
Party Drugs,& Group Sex- (M)	91	**18.7**	**5.3 (2.6 to 10.8)**

Modified & Adapted From: I. B. Zablotska, G. Prestage *et al*., JAIDS **62(3):**334-338,2013.

Informal HIV Pre-exposure Prophylaxis (PrEP) With Australian Homosexual Men-Cont'd

- Informal/improper drugs involved:
  - Using what drugs unknown
  - Injecting what drugs unknown
  - Using party drugs for enhancing sex
  - Group sex after or while on party drugs
  - Other
- Lack of antiviral information as to:
  - Which antivirals used for PrEP and/or PEP

Informal HIV Pre-exposure Prophylaxis (PrEP) With Australian Homosexual Men-Cont'd

  - Where antivirals obtained
  - How often antivirals taken
  - How long antivirals used
  - What antiviral doses used
- HIV increased infection rates are mostly driven by non-protected anal sex.
- PrEP adherence levels often are low.

Pre-Exposure Prophylaxis Comments/Thoughts/Questions

- “Another intervention that distracts from ensuring safer sex”
- ‘A potentially effective tool, but if used incorrectly or inconsistently becomes a worthless tool’
- “A pill does not always prevent or cure”