LABORATORY DIAGNOSIS OF HIV INFECTIONS – AN UPDATE

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INTRODUCTION

• HIV - etiologic agent of AIDS
• 36.9 million infected worldwide; 2 million new cases; 15 million on ART (2014)
• 1.2 million deaths - 4th leading cause of death worldwide; 34 million overall (2014)
• 2nd leading cause of death in young adults
• Progressive deterioration of immune system; usually fatal if untreated
GLOBAL PREVALENCE

Adults and children estimated to be living with HIV | 2011

Total: 34.2 million [31.8 million – 35.9 million]

UNAIDS. Report on the Global AIDS Epidemic; 2012
HISTORICAL BACKGROUND

- First well-documented case dates to 1959 in a African man
- First clinically recognized in 1981
- Unexpected cluster of diseases including Kaposi’s sarcoma & PCP in young homosexual men
- Patients noted to have depletion of CD4 positive helper T-cells
HISTORICAL BACKGROUND

- AIDS subsequently reported in IVDU, hemophiliacs, infants born to mothers w AIDS
- Suggests blood-borne and sexually transmitted pathogen
- 1983 - HIV-1 isolated; novel human retrovirus
- 1984 - AIDS reportable disease
- 1985 - HIV-1 antibody test developed; zidovudine successfully used, genome sequenced
ORIGINS OF AIDS

• HIV strains arose from non-human primate lentiviruses
• West Africans prostitutes had Ab more reactive to SIV than HIV
• HIV-2 isolated (1986); more closely related to SIV than HIV-1
ORIGINS OF AIDS

• SIVsm - sooty Mangabey monkeys; closely related to HIV-2
• SIVcpz - chimpanzee; closely related to HIV-1
• Range is West Equatorial Africa
• 3 genetically diverse HIV-1 groups: main (M), outlier (O), non-M, non-O (N)
ORIGINS OF AIDS

Ancestral Lentiviruses

- HIV-2
- SIV_{SM}

- SIV_{SY}

- HIV-1
  - SIV_{CPZ}
  - SIV_{AG}
VIROLOGY

- Enveloped, ssRNA virus
- Family: *Retroviridae*; Genus: *Lentivirus*
- Reverse transcriptase makes a DNA copy (cDNA) from RNA genome
- Integration into host genome as provirus
- Cytopathic, non-oncogenic, slowly developing disease
VIROLOGY

- Three distinct genetic groups of HIV-1; M, O, and N; gag & env sequence diversity
- Group M (Major) - predominates; genetic subtypes or clades; A-D, F-H, J & K; some recombinants; B clade major US type; C clade dominates globally
- Group O (Outlier) - rare; Cameroon, Gabon, Equatorial Guinea
- Group N (non-M, non-O) - Cameroon
- HIV-2 - 7 subtypes (A-G); A & B dominate; West Africa
VIROLOGY

GROUP M

G H J

GROUP N

SIV_{CPZ-GAB} SIV_{CPZ-US} SIV_{CPZ-CAM3}

GROUP O

in env

SIV_{CPZ-ANT}

OTHER PRIMATE LENTIVIRUSES

0.10
GLOBAL DISTRIBUTION OF HIV-1 SUBTYPES
VIROLOGY

• Viron - 100-150 nm w/ lipid envelope & core containing 2 copies of ssRNA (10 kb)

• Genes
  – gag (structural proteins)
  – pol (viral enzymes)
  – env (envelope glycoproteins)
  – LTR & ORF
VIROLOGY

- **gag** - p55 (precursor); p17-matrix, p24-capsid, p7-nucleocapsid
- **pol** - p170 (precursor); p11-protease, p32-integrase, p66/p51-RT/RNase
- **env** - gp160 (precursor); gp41-transmembrane, gp120-surface
MODE OF TRANSMISSION

• Two exclusive modes
  – Sexual transmission
  – Transfer of blood, blood products, body fluids

• Similar to HBV – doesn’t survive as long outside host; more susceptible to heat & disinfectants

• Must cross epithelial cell barrier into fluid compartments

• Genital ulcers - herpes, syphilis, chancroid; increased susceptibility due to break in mucous membranes
MODE OF TRANSMISSION

• High levels of free HIV & infected leukocytes; mucous membrane or percutaneous transfer = significant risk
  – Blood
  – Semen
  – Vaginal secretions
  – Breast milk (leukocytes)

• Low levels of HIV (< 1/mL) = little/no risk
  – Sweat, tears
  – Urine
  – Saliva
EPIDEMIOLOGY - USA

- 1.2 million cases (2013)
- New cases – stable since early 2000
- ~50,000 new cases/yr; 47,352 (2013)
- ~15,000 deaths/year; 13,712 (2013)
- 658,507 deaths since beginning of pandemic through 2013
- New therapies - lengthened lives and slowed death rate
HIV Incidence by Year - USA

- People living with HIV
- New HIV infections — back-calculation
- New HIV infections — incidence surveillance methodology

Hall JAMA 2008; Prejean Plos One 2011; MMWR 2011
EPIDEMIOLOGY - USA

- AIDS still 8th leading cause of death in men 24-44 years old (2011)
- Males (79%) > females (21%); blacks (46%) > whites (29%) > hispanics (20.5%)
- Age: ≤ 14 (0.4%); 13-24 (26%); 25-34 (31%); 40-64 (38.0%); ≥ 65 (1.9%)
- Majority of cases in large metropolitan centers of NY, CA, FL, DC, TX
HUMAN IMMUNODEFICIENCY VIRUS DIAGNOSES. Diagnosis rates* — United States and U.S. territories, 2012

* Per 100,000 population.
RISK GROUPS

- MSM – 63% of new cases – increasing
- Heterosexual partners of HIV carriers – 25% of new cases
- IVDU – 8% of new cases
- Perinatal – 127 (0.25%) new cases
- Other* – 65 (0.13%) new cases
**EPIDEMIOLOGY - USA**

- **Congenital/Neonatal**
  - 127 cases in 2011 – continues to decrease
  - HAART can prevent infection
- **Blood products** - no longer a serious risk
  (1 in 1.5 million; 2010 estimate)
- **HCW** – a few dozen cases
- **Unknown/Inapparent risk** - <0.1% of cases
EPIDEMIOLOGY - Africa

- Females > Males
- Primary mode of transmission - heterosexual contact (90%)
- 1.8 million new infections (2011)
- 23.5 million living with HIV (2011)
- Mortality – 1.2 million (2011); 71% of global HIV related deaths
EPIDEMIOLOGY - Others

- Pandemic involves Europe, Latin America, India & SE Asia
- Eastern Europe & former Soviet Union – 1.5 million cases (2011)
- Asia – 4.8 million cases; 440,000 new cases; 309,000 deaths (2011)
- Latin America/Caribbean – 1.4 million cases; 83,000 new cases (2011)
PATHOLOGY

- HIV entry - dendritic cell infection
- Replication and shedding
- Macrophage infection in skin, lymphatics, bone marrow, blood
- Progressive destruction of helper T-lymphocytes, monocytes, macrophages, B-lymphocytes
PATHOLOGY

• Glycoprotein on HIV surface (gp120) binds to CD4 & CCR5 (macrophages) or CXCR4 (T-cells) receptors on cell surface – docking & fusion

• Within cell - reverse transcriptase (RT) makes cDNA from ssRNA genome

• Initially lytic infection followed by latency and integration of viral DNA into host cell genome (via integrase) - provirus
PATHOLOGY

- Immune activators stimulate latent cell
- Transcription of proviral DNA - mRNA
- Translation of mRNA into capsid, RT & other viral proteins
- Viral assembly, budding, cell lysis
PATHOLOGY

• Lysis of T-cells & other leukocytes - leukopenia, loss of essential memory clones & stem cells

• Formation of syncytia - spread of virus from cell to cell; syncytia lysis

• Viral load increases, cell infection & lysis increases, even greater viral load
PATHOLOGY

• Destruction of CD4 lymphocytes - loss of helper, cytotoxic, inducer functions
• CD4 cells < 200/µL - AIDS
• Paves way for cancer & invasion by opportunistic pathogens
• Also cytotoxic for CNS - glial & other cells; peripheral nerve demyelination; encephalitis
CLINICAL MANIFESTATIONS

- Incubation phase - 1-2 weeks
- Vague mononucleosis-like syndrome - “acute” HIV infection
- Fever, rash, oral ulcers, night sweats, lymphadenopathy, fatigue, diarrhea, weight loss, arthralgia, pharyngitis
- Asymptomatic – years (10)
- Non-progressors – 5-10%; deletion of CCR5 receptor
CLINICAL MANIFESTATIONS

• With continuing loss of CD4+ cells – progression to AIDS (AIDS defining illness)
  – Opportunistic infections, e.g. PCP
  – Cancers (KS, B-cell lymphomas, others)
  – Wasting syndrome
  – Neurologic - AIDS dementia
TREATMENT

• Six classes of anti-retroviral therapy
  – Nucleoside RT inhibitors (NRTI)
  – Nonnucleoside RT inhibitors (NNRTI)
  – Protease inhibitors (PI)
  – Fusion inhibitors (FI)
  – Entry inhibitors (EI)
  – Integrase inhibitors (II)

• HAART - 2 NRTI + NNRTI (or PI or FI)

• Dramatic effect on disease progression, death, quality of life
ACUTE HIV INFECTION

• Acute HIV infection (AHI) – time between positive viral load and seroconversion
• Very high viral loads in genital secretions and blood; persists for 10-12 weeks
• Rate of transmission 26 times higher vs established HIV infection
• May account for 10-50% of all new HIV infections
LABORATORY DIAGNOSIS

• Diagnosis of acute HIV infection
  – Improve understanding of transmission
  – Early intervention may limit replication & integration to the latent (incurable) state
  – Maximally contagious
  – Early diagnosis - opportunities for treatment and prevention interventions
    • Routine screening
    • Use of short time to positivity (TTP) tests
LABORATORY DIAGNOSIS

• Three major method categories
  – Virologic methods – culture
  – Serologic methods – antibodies & antigens
  – Molecular methods - e.g. PCR

• Symptomatic & high-risk - straightforward

• Asymptomatic & low-risk - more complex

• Adverse economic, social, psychological cost of inaccuracies; need tests with high sensitivity & specificity
VIROLOGIC METHODS

• Virus Isolation
  – Plasma or PBMC
  – Provides direct evidence of HIV-1 infection
  – Sensitivity $\geq 95\%$ if CD4 < 500/µL
  – Sensitivity lower if CD4 > 500/µL
  – Rarely necessary to establish diagnosis
  – Value limited to research purposes
SEROLOGIC METHODS

• Antigen Detection
  – Presence of p24 (capsid) detectable by EIA
  – High titers in acutely infected patients prior to seroconversion; day 7-14
  – After seroconversion, complexed w/ p24 antibody - undetectable
  – Later appearance of p24 - clinical progression
  – Detect ≥ 4000 virons
  – Interfering substances - false-positive; neutralization
SEROLOGIC METHODS

• Most cases – antibody HIV-1/HIV-2

• Seroconversion
  – 3rd gen Ab tests - mean 22-25 days
  – 4th gen Ag/Ab tests – 5-10 days sooner (sens 99.9%; spec 98.8%)

• Two-stage process
  1) Repeatedly reactive screening test
  2) Confirmatory assay
SEROLOGIC METHODS

HIV Ab EIA

• Antigen preparations - 3 types
  1) 1\textsuperscript{st} gen - infected T-cell line lysates
  2) 2\textsuperscript{nd} gen - recombinant proteins
  3) 3\textsuperscript{rd} (& 4th) gen - recombinant antigens and oligopeptides
SEROLOGIC METHODS

HIV Ab EIA

- 1st Generation Tests
  - Biologic false + w/ lysate antigen due to Ab to HLA proteins expressed in lymphoid cell lines
  - Ab commonly found in multiparous women & patients who have had multiple transfusions
  - Mean time to seroconversion – 50 days
SEROLOGIC METHODS

HIV Ab EIA

• 2nd Generation Tests
  • No cross reaction w/ HLA proteins
  • Included HIV-2
  • Biologic false + (fewer) due to cross reaction w/ yeast or bacterial antigens (recombinant)
  • May fail to detect Ab to highly divergent HIV-1 subtypes from groups N & O
  • Mean time to seroconversion – 37 days
SEROLOGIC METHODS

HIV Ab EIA

• 3rd Generation Tests
  – Ag sandwich EIA
  – Detect all classes of Ab; groups N & O
  – Greater sensitivity in early infection – mean time to seroconversion – 22 days

• 4th Generation Tests – p24/Ab combination; earlier diagnosis – 15-16 days
SEROLOGIC METHODS

HIV Ab 3rd & 4th Gen EIA

• Sensitivity ≥99.5%
• False negatives - absence/low level Ab (or Ag) in early primary infection (0.02-0.3%)
• Specificity ≥99%
• Fewer false positives
• Low prevalence (0.5%); PPV = 50%
SEROLOGIC METHODS

HIV Ab - Rapid tests

• Membrane EIA & immunochromatographic; oral and blood specimens
• Simple; sensitivity & specificity comparable in ideal conditions but lower in field; skill level
• Several test kits available including waived
• New 4th gen rapid tests include Ag detection
• More expensive; longer TTP; group N
• Parturient women, needle stick injuries
• If reactive, must enter new algorithm
SEROLOGIC METHODS

Western Blot
- In low prevalence population - low PPV
- Use of WB as confirmatory test “essential” to exclude false positive results – old algorithm
- Detection of Ab to individual proteins
- Ab to Gag proteins (p17, p24, p55) appear earliest (30 days); decrease with progression
- Ab to Env proteins (gp160, gp120/gp41) appear later but persist even w/ advanced AIDS
**Western Blot**

- Coordinate use - EIA/WB ~ 100% specific
- Criteria for +WB
  - CDC/ASTPHLD - any 2 bands of p24, gp41, or gp120/160
  - WHO - ≥ 2 env proteins
  - ARC - ≥ 1 band in gag, pol & env
- No bands = negative; ≥ 1 band but not meeting positive criteria = indeterminate
HIV-1 Western Blot
SEROLOGIC METHODS

Interpretation of HIV Serology – Old Algorithm
• Repeatedly + EIA and + WB = HIV-1 infection
• Repeatedly + EIA and negative or ind. WB
  – False + EIA
  – Recently acquired HIV infection - within 4-5 wks
  – HIV-2 infection - perform HIV-2 WB if suspected
  – Groups other than M - variable results

• Negative EIA
  – Absence of HIV infection provided no high-risk behavior or ≥ 6 months since exposure
  – Recently acquired HIV infection - within 6 mos
OTHER CONFIRMATORY ASSAYS

- Multispot® - differentiates HIV-1 from HIV-2
- Indirect Immunofluorescence – screen or confirmatory assay
- Line Immunoassay – similar to WB; antigens placed on strip
- HIV-1 NAT
  - APTIMA HIV-1 RNA Qual (Hologic Gen-Probe)
  - Procleix Ultrio Procleix (Novartis)
ALGORITHM ISSUES

• Focus change from “specificity” to “sensitivity”
• Time to positivity (TTP) of tests; acute HIV
• 4th generation tests (4GT)
  – Earlier TTP (14-16d); close to HIV RNA VL (8-10d)
  – 89% of VL+/Ab- → 4GT+
  – Cheaper than VL - $50 vs $6
• WB long TTP – 25-35 days; no longer part of HIV testing algorithm
• Multispot® – differentiation/confirmatory test
SEQUENCE OF LAB MARKERS

HIV RNA (plasma)

HIV-1 p24 Antigen

HIV Antibody

HIV Infection

Days

Eclipse Period

Acute HIV Infection

Established HIV Infection

Viral Detection

Nucleic acid test

3rd generation Immunoassay

2nd generation Immunoassay

1st generation Immunoassay

Seroconversion window

CDC: June 27, 2014
LABORATORY STAGING

Natural History and Laboratory Staging of HIV Infection

Eclipse Phase

I  II  III  IV  V  VI

(v RNA+)

(p24Ag+)

(ELISA+)

(Western blot +/−)

(Western blot + (p31−))

(Western + (p31+))

Plasma virus RNA (copies/ml)

Days following HIV-1 transmission

Viral RNA cutoff 50 copies/ml

Ultrasensitive Viral RNA cutoff 1-5 copies/ml

(adapted from Fiebig, AIDS 2003)
TIME TO POSITIVITY

Days following HIV Infection

HIV Tests

HIV RNA VL +

Eclipse Phase

P24 +

HIV Ab + (EIA)

HIV WB +/-

HIV Ab + (POC)

HIV WB +
FIGURE 2. Reactivity of FDA-approved assays for HIV-1 compared with Western blot.
NEW ALGORITHM

- 4th generation HIV Ag/Ab assay – initial test
- If reactive, HIV Ab differentiation assay – confirmatory test
- Western Blot - discontinued as confirmatory assay
- If differentiation test negative, perform HIV NAT; if NAT positive - acute HIV infection
- If initial use of rapid HIV is reactive, enter new algorithm from beginning

CDC: June 27, 2014
Box 1. Recommended Laboratory HIV Testing Algorithm for Serum or Plasma Specimens

HIV-1/2 antigen/antibody combination immunoassay

- (+)
  - HIV-1/2 antibody differentiation immunoassay
    - HIV-1 (+)
      - HIV-1 antibodies detected
    - HIV-2 (-)
      - HIV-1 antibodies detected
    - HIV-1 (-)
      - HIV-2 antibodies detected
    - HIV-1 (+)
      - HIV antibodies detected
    - HIV-1 (-) or indeterminate
      - HIV-2 (-)
        - HIV-1 NAT
          - HIV-1 NAT (+)
            - Acute HIV-1 infection
          - HIV-1 NAT (-)
            - Negative for HIV-1

- (-)
  - Negative for HIV-1 and HIV-2 antibodies and p24 Ag

If high risk, consider

(+)) indicates reactive test result
(-) indicates nonreactive test result
NAT: nucleic acid test

CDC: June 27, 2014
SEROLOGIC DIAGNOSIS OF HIV-2

- Early HIV-1 Ab EIA (1985-95) - sensitivity for HIV-2 - 60-90%
- Early-mid 1990’s - HIV-1/HIV-2 EIAs
- 3rd & 4th gen HIV-1/HIV-2 Ab EIAs - ≥99.5% sensitive for both
- Multispot – immunoassay which differentiates HIV-1 from HIV-2
- Need HIV-2 specific WB & molecular assays
- Very low prevalence in USA - no routine testing (CDC); blood banks; if indicated
SEROLOGIC DIAGNOSIS OF HIV-2

- Prevalence high West Africa
- HIV-1 WB may be positive in HIV-2
- “Cryptic” HIV-2 infection
- Clinical deterioration when treating for HIV-1 despite undetectable HIV-1 viral loads because HIV-2 does not respond to NNRTI or several protease inhibitors
MOLECULAR METHODS

Qualitative Proviral DNA PCR

- Presence of proviral DNA in PBMC
- PCR targets *gag* or *pol* genes
- Near 100% sensitivity/specificity in experienced lab; 96-99% in field
- Predicted sensitivity of 99% when ≥ 10 DNA copies present
- Limited utility - infection in neonates; at birth, 4-7 wks, and 8-16 wks
MOLECULAR METHODS

Qualitative TMA

- Sensitivity - 30 copies/mL plasma; specificity 99.8%
- Early diagnosis - 6 days before p24
- Acute HIV infection
- Blood donor screening
- Diagnosis in the neonate
- Confirmatory test; resolve ind WB
MOLECULAR METHODS

Quantitative Plasma RNA (Viral Load)

- Monitor disease progression, **response to therapy**, infectiousness
- Several methods - RT-PCR, NASBA, TMA, bDNA; all highly correlated
- Lower limit - 20-50 copies/mL
- Early generation assays - low precision < 200 copies/mL; better now
MOLECULAR METHODS

HIV Viral Load

• 1st gen RT-PCR assays - limited sensitivity for detection & quantification of non-B subtypes

• bDNA superior for A, E & F subtypes; but lower viral loads overall & required higher specimen volume

• 2nd/3rd gen RT-PCR assays - lower LOD; detects all M, N & O groups
MOLECULAR METHODS

HIV Viral Load

- Interassay variability varies inversely with plasma conc. - 0.038-0.23 log_{10} copies/mL
- Serial testing in stable patients not on ART over wks-mos - biological variation 0.3 log_{10}
- Changes of > 0.5-0.7 log_{10} copies/mL (3-5-fold) likely reflect significant changes in HIV replication
Clinical Utility of Viral Loads

- Plasma HIV RNA levels correlate with stage of disease (CD4+ count also)
- High titers - symptomatic disease/AIDS
- Low titers - asymptomatic
- >100,000 copies within 6 mos of sero-conversion - 10X more likely to develop AIDS within 5 years
MOLECULAR METHODS

Clinical Utility of Viral Loads

• Assess effectiveness of HAART
• Decrease in HIV RNA - reduction in risk of disease progression
• $0.3 \log_{10}$ decrease - 30% reduced risk of progression to AIDS/death; $1 \log_{10} = 67$
• HIV RNA & CD4+ cell count - important prognostic indicators
Therapeutic Monitoring

First phase ($t_{1/2} = 1$ day)

Second phase ($t_{1/2} = 14$ days)

Third phase? ($t_{1/2} =$ ?)

Limit of detection

Plasma HIV-1 RNA (copies/ml) vs. Time on HAART (days)

Cell 1998; 93:665-71
MOLECULAR METHODS

Clinical Utility of Viral Load

• Immune activation - transient rise in viral load
• For individual patient - use same lab
• Two baselines recommended prior to HAART
• Repeat 2-8 weeks after start of HAART
• Expect decline of $2 \log_{10}$ within 8 weeks (HAART naïve pt)
• Repeat every 3-4 months
MOLECULAR METHODS

HIV Viral Load for Dx of $1^0$ Infection

- Not FDA approved for this purpose
- Controversial - sensitivity $\geq 99\%$; specificity 97.4%
- False + usually $< 2,000$ copies/mL
- True + usually $> 5,000$ copies/mL
- Screening of donated blood or HIV Ab negative - pooled
DRUG RESISTANCE TESTING

• Genotypic – mutations associated with drug resistance
• Phenotypic – viral susceptibility to specific drugs (IC₅₀)
• PhenoSense – target genes from patient HIV amplified & inserted into vectors; recombinants used in culture
DRUG RESISTANCE TESTING

• Genotypic assays
  – Advantages
    • Fast & easy
    • Low cost
    • Detects resistance earlier
  – Disadvantages
    • Resistant variants >25-30%
    • Plasma RNA ≥ 1000 copies/mL
    • Mutations may not correlate with phenotype
    • Cross resistance
DRUG RESISTANCE TESTING

• Phenotypic assays
  – Advantages
    • Susceptibility data in familiar format ($\text{IC}_{50}$)
    • Demonstrate resistance in absence of genetic basis
  – Disadvantages
    • Resistant variants >25-30%
    • Availability
    • Cost
DRUG RESISTANCE TESTING

Indications

• Selection of initial therapy
• Regimen switch
  – Risk of failure reduced 30-50%
  – Greater decreases in viral load
• Treatment failure & pregnant women
• Areas w/ high prevalence of resistance
TROPISM ASSAY

- HIV entry into target cells through receptors CCR5 and/or CXCR4
- If CCR5 antagonists, e.g. maraviroc, blocks receptor, prevents entry and infection
- Used in treatment if CCR5 primary receptor
- Tropism assay makes recombinant HIV from patient’s virus, then determines ability to enter cells with receptors and with and without antagonist
SUMMARY

• HIV infection highly prevalent worldwide
• Identifying “acute” HIV infection important for effective intervention
• HIV tests have evolved and are positive earlier in the course of infection
• Testing focus has changed from “specificity” to “sensitivity” – new algorithm
• Tests for therapy selection and monitoring
LABORATORY DIAGNOSIS OF HIV INFECTIONS – AN UPDATE

Questions?
Comments?

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