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HIV antiretroviral drug resistance

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Failure of antiretroviral therapy (ART)

Possible causes

- poor adherence (compliance)
- pharmacokinetic factors
  (e.g. ↓ absorption from gut)
- changes in drug metabolism
  (e.g. due to anti-tuberculosis co-medication)
- Drug failure sensu stricto:
  emergence of antiretroviral drug
  resistant HIV strain(s)
Generation of diversity in HIV-1 during the viral replication cycle

Reverse transcription results in approximately 1 substitution and 7 to 30 crossovers per genome per round of replication

Approximately $10^{10}$ virions produced per day per person

Millions of variants per day
Emergence of resistant virus due to selective drug pressure

Drug-sensitive virus

Drug-resistant virus

No antiretroviral treatment

HIV viral load

Time
Emergence of resistant virus due to selective drug pressure

Fully suppressive antiretroviral treatment

- Drug-sensitive virus
- Drug-resistant virus

HIV viral load vs. Time
Emergence of resistant virus due to selective drug pressure

- Drug-sensitive virus
- Drug-resistant virus

Selective drug pressure
Evolutionary bottle-neck

HIV viral load vs. Time
Antiretroviral drug resistance in clinical practice

• Acquired resistance = resistance developing within an infected patient due to:
  • exposure to PMTCT (esp. sdNVP-only)
  • retention on failing ART
  ⇒ accumulation of resistance mutations
  ⇒ chance of transmission

• Primary resistance = de novo infection with resistant HIV strain
  • 10 – 20 % of cases in Europe, USA
  ⇒ current guidelines recommend resistance testing prior to start of ART
Research on antiretroviral drug resistance at Tygerberg

- Study patients attending the Family Clinic for HIV at Tygerberg Academic Hospital and other clinics in the Western Cape
- Identify ARV drug resistance in:
  - PMTCT-exposed women and children
  - Adults and children failing HAART
  - Untreated patients (transmitted resistance?)
- Document factors associated with resistance
- Improve laboratory techniques
Resistance following PMTCT

- > 95% of pregnant women tested
- PMTCT regimen:
  mother: ART or AZT from 34th week + sdNVP;
  baby: sdNVP plus AZT for 7 days
- Transmission rate <5%
- Risk of maternal NVP resistance: 17.1%
- For comparison: sdNVP-only programmes
  ~ 35.7% (range 25 – 69%)
- Dual PMTCT only recently introduced nationwide!

Antiretroviral therapy (ART) in South Africa: public health approach

- pre-2010 ART guidelines for adult patients:

  - d4T + 3TC + EFV or NVP
  - AZT + ddI + LPV/r

  Failure (VL > 5000 cop/ml) high!

  toxic!
Acquired resistance in adults failing 1st line ART: materials & methods

- 167 patients (115 ♀, 52 ♂) failing 1st line ART:
  - 136 on D4T, 28 on AZT and 3 on Tenofovir
  - all but two on 3TC
  - 85 on NVP, 83 on EFV
Acquired resistance in adults failing 1st line ART: results

- 17% no resistance-associated mutations
- 82% NNRTI resistance
- 60% M184V/I mutation (conferring 3TC resistance but reducing viral 'fitness')
- 7 patients (4%) had K65R
- 4 patients (2.3%) had ≥3 TAMs
- Patients with ≥3 TAMs had been failing the regimen for a significantly longer duration than those without

van Zyl et al., J Med Virol 2011
13

Pre-2010 adult 1st line ART regimen: stepwise failure

Accumulation of TAMs (d4T and AZT resistance)

3TC resistance

NNRTI resistance (NVP, EFV)

5000 copies / ml

viral load threshold for therapy switch

viral load test done every 6 months

log viral load

months on failing regimen

0 1 2 3 4 5 6 7 8

6 5 4 3 2 1 0

6
Antiretroviral therapy (ART) in South Africa: public health approach

- 2010 revised ART guidelines for adult patients:
  
  \[
  \text{TDF or AZT} + \text{ 3TC or FTC} + \text{ EFV or NVP}
  \]

  Failure \( (\text{VL} > 1000 \text{ cop/ml} \times 2) \)

  \[
  \text{AZT or TDF} + \text{ 3TC or FTC} + \text{ LPV/r}
  \]

  Failure \( (\text{VL} > 1000 \text{ cop/ml} \times 2) \)

  Specialist referral
Acquired resistance in adults failing 2nd line ART

- 93 patients on LPV/r-based 2nd-line regimen
- 37/93 (39.8%) failing ART (VL>500 copies/ml)
- Resistance testing successful in 33 patients
  ⇒ Only 2/33 had major PI resistance mutations
- Drug exposure over time measured via LPV plasma and hair levels
  ⇒ Low hair or low random plasma levels had high negative predictive value for virological failure (96% and 92%, resp.)
  ⇒ The problem is poor adherence rather than drug resistance

van Zyl et al., J Acquir Immune Defic Syndr 2011
Using hair to monitor drug exposure in patients on ART

van Zyl et al., J Acquir Immune Defic Syndr 2011
*Collect plasma specimens for lopinavir concentration and HIV-1 RNA Load and concurrent occipital hair specimen

Analyse HIV-1 RNA Load

< 50 copies/ml

Continue therapy; Routine management

50 copies < HIV RNA load < 1000 copies/ml

LPV plasma < 1 μg/ml OR LPV hair < 3.63 ng/mg

Investigate and address adherence issues; Follow-up specimen collection* in 3-6 months

> 1000 copies/ml

Analyse LPV hair and plasma concentration

LPV plasma > 1 μg/ml AND LPV hair > 3.63 ng/mg

Genotypic antiretroviral resistance testing
Initial drug resistance in children

- Initial DR = transmitted + PMTC-induced DR
- Adapted WHO HIV Drug Resistance Threshold Survey method to classify TDR into categories: low (≤ 5%), moderate (5%–15%), or high prevalence (≥ 15%)
- 49 ART-naïve infants <18 months included
- 35/49 children not exposed to PMTCT (= sd-NVP + short-course AZT)
- 48 RT and 49 PI sequences obtained

van Zyl et al., Paed Infect Dis J 2010
Initial drug resistance in children

- No NRTI or PI resistance mutations detected
  \[ \Rightarrow \] prevalence classified as < 5%
- 2 x K103N and 1 x V90I identified
  \[ \Rightarrow \] prevalence classified as 5 – 15%

- Primary interest in NRTI and PI resistance as it could signify transmitted resistance
- NNRTI-associated mutations induced by PMTCT detected in 3 patients by “bulk sequencing”
- Predicts good therapeutic response to currently used regimen in infants in the Western Cape

van Zyl et al., Paed Infect Dis J 2010
Introduction:
Transmitted HIV drug resistance (TDR) can compromise initial antiretroviral therapy (ART), resulting in early therapy failure despite good adherence. In a population with a high prevalence of TDR, early or baseline resistance testing may be necessary to determine an optimal, individual ART regimen, which is not feasible in resource-constrained settings. We evaluated the prevalence of TDR in recently HIV-infected adults in the Western Cape province, South Africa, using the World Health Organization’s threshold surveillance method.

Methods:
We included specimens sent consecutively for CD4 counts to the Tygerberg Laboratory if they fulfilled the following criteria: patient >15 years old; CD4 count <500 cells/μL; not on ART according to request form. After anonymization, population sequencing was performed. Sequences were interpreted using the calibrated population resistance (CPR) test of the Stanford University database according to the updated WHO surveillance drug resistance mutation (SDRM) list.

Results:
Specimens included were from 46 females and 1 male, median age 18 years (range 15-20 years); median CD4 count 355 cells/μL (range 50 – 2500 cells/μL) were included, of which 40 (89%) were successfully amplified and sequenced. The survey was discontinued thereafter based on the absence of SDRM mutations; using the WHO threshold analysis classification, this result predicts a low (~1%) prevalence of TDR to all three drug classes in this population. The TDR resistance-associated polymorphisms were detected in two samples. This polymorphism is frequently observed in HIV-1 subtype C and together with other primary resistance mutations causes resistance to certain protease inhibitors.

Conclusions:
According to the WHO SDRM list, TDR was detected in this survey, suggesting a low prevalence (~1%) of TDR in the Western Cape province four years after the public sector ART roll-out programme was started. While this is encouraging, ongoing vigilance is required to ensure the continued success of the programme.

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Conclusions

- Acquired resistance likely to $\uparrow$ due to increasing numbers of people on ART and late detection of failure
- Primary (transmitted) resistance will also eventually $\uparrow$

$\Rightarrow$ Ongoing resistance monitoring & surveillance needed!

- We must also maintain our comparatively high standard of monitoring patients on ART incl. regular 'viral load' testing
Limitations of current genotypic testing by population sequencing

- Complicated and expensive
- Lacks minority population sensitivity
- Imperfect interpretation algorithms
- Clinical interpretation complex, must take into account:
  - treatment history (archived resistance?)
  - current adherence
  - drug levels
  - any previous resistance test results
The future of resistance testing

- Time for recommendations: Is there a role for resistance testing in the public sector?
- More affordable and simpler assays; e.g.
  - Allele-specific assays (e.g. TaqMan PCR)
    - allelic discrimination targeting "signature mutations"
  - detection of minority species
  - might replace viral load testing at the same time
- Next generation (deep) sequencing
  - Detects minority variants: relevance?
  - High coverage might allow pooled testing of specimens through "barcoding"
Allele-specific TaqMan PCR

**Figure 3.9:** An amplification plot showing the discriminatory ability of the K103N-specific SPCR assay on all 12 genetically varying standards to ensure the specificity of K103N-specific primers. The plot includes amplification curves for all three K103N-mutant standards encoded by AAT (MS3-1, MS3-2, and MS3-4). Four amplification curves for K103N-mutant standards encoded by AAC (MS10-3, MS10-4, MS15-3, and MS15-4). Five amplification curves for the five K103-wildtype standards (MS9-2, MS9-3, MS9-4, MS10-1, and MS10-2).
Massive Parallel Sequencing
Hepatitis B virus infection and HIV

- HBsAg prevalence 5 – 10%
- No routine HBsAg screening in pregnancy or before ART
- HBV routine immunisation (since 1995): 6 – 10 – 14 weeks
- ART first-line (until 2010): Stavudine + Lamivudine (3TC) + NNRTI

⇒ HIV first-line therapy means automatically monotherapy against HBV!

SA Dept. of Health Guidelines
Antiviral drug-associated potential vaccine escape mutant HBV (ADAP-VEM)
Thank you, baie dankie, enkosi kakhulu, vielen Dank!