Growing up with HIV

Metabolic Complications of HIV and its treatment in children

Alessandra Viganò, MD
Pediatric Clinic, L. Sacco Hospital
University of Milan
Thank you

Colleagues and Coworkers

<table>
<thead>
<tr>
<th>G Aldovrandi</th>
<th>V Giacomet</th>
</tr>
</thead>
<tbody>
<tr>
<td>C Cerini</td>
<td>V Manfredini</td>
</tr>
<tr>
<td>C Mameli</td>
<td>S Mora</td>
</tr>
<tr>
<td><strong>G Bedogni</strong></td>
<td>G.V Zuccotti</td>
</tr>
<tr>
<td><strong>C. Gabiano</strong></td>
<td></td>
</tr>
</tbody>
</table>

The European Pediatric HIV and lipodystrophy study team:

<table>
<thead>
<tr>
<th>C Thorne</th>
<th>NM Alan</th>
</tr>
</thead>
<tbody>
<tr>
<td>M Cortina-Borja</td>
<td>T Goetghebuer</td>
</tr>
<tr>
<td>M Marczynska</td>
<td>R Rosso</td>
</tr>
<tr>
<td>S Bernardi</td>
<td>F Salvini</td>
</tr>
<tr>
<td>A Guarino</td>
<td>A Maccabruni</td>
</tr>
<tr>
<td>L Galli</td>
<td>R Badolato</td>
</tr>
<tr>
<td>C Giaquinto</td>
<td></td>
</tr>
</tbody>
</table>

...and all children and families
## Longer Exposures

<table>
<thead>
<tr>
<th></th>
<th>&lt;12m</th>
<th>1 to &lt;3 years</th>
<th>3 to 5 years</th>
<th>≥5 yrs</th>
</tr>
</thead>
</table>
| **US**     | ALL  | • Significant Sx (CDC B & C except LIP or single episode of serious bacterial infection)  
• CD4 <25% regardless of Sx | • CDC B & C or  
• WHO 3 & 4  
• CD4 <25% or <1000 cell/mm³ | • Significant Sx  
• CD4 <350 cell/mm³ |
| **PENTA**  | ALL  | • CDC B & C or  
• WHO 3 & 4  
• CD4 <25% or <1000 cell/mm³ | • CDC B & C or  
• WHO 3 & 4  
• <20% or <500 cell/mm³ | • CDC B & C or  
• WHO 3 & 4  
• CD4 <350 cell/mm³ |
| **WHO**    | ALL  | • WHO Stage 3 & 4 if no CD4 count;  
• with CD4, Stage 3 (TB, LIP, OHL, ↓ platelets) only if CD4 <750 or <20% | • WHO Stage 3 & 4 if no CD4 count;  
• with CD4, Stage 3 (TB, LIP, OHL, ↓ platelets) only if CD4 <350 or <20% | • WHO Stage 3 & 4 if no CD4 count;  
• with CD4, Stage 3 (TB, LIP, OHL, ↓ platelets) only if CD4 <200 or <15% |
|            |      | WHO Stage 1 or 2 only if CD4 <750 or <20% | WHO Stage 1 or 2 only if CD4 <350 or <20% | WHO Stage 1 or 2 only if CD4 <200 or <15% |
Exposure at Critical Times

Weight gain, kg per year

Birth to 4-6m: 2 x birth wt
Birth to 1 yr: 3 x birth wt
By 2 yrs: 4 x your birth wt
1/2 your adult ht

Girls
Boys

Aich Dis Child 1986; 41: 454-471

Age, years

Lymphoid

Each curve plots the size of a group of organs or body parts as a % of their size at age 20 (which is the 100% level on the vertical scale).

Brain & head
General
Reproductive

Adapted from Growth and Adolescence
2nd Ed by JM Tanner, 1962

Size attained as % of total potential growth

Height gain, cm/yr

Girls
Boys

Age in years

Arts: Dis Child 1986; 41: 454-471

5
10
15
20

15
10
5

0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200

16-17 July 2010, Vienna Austria
Objectives

- Briefly review data on morphologic and metabolic abnormalities described in perinatally HIV-infected children

- Focus on cardiovascular and bone abnormalities

- Emphasis on unique aspects of pediatric HIV infection
Alteration in Body Fat

• Lipoatrophy
• Lipohypertrophy
• Combined lipodystrophy
Alteration in body fat

Presented at the 2nd International Workshop on HIV Pediatrics
16-17 July 2010, Vienna Austria
Lipodystrophy syndrome

- Prospective data on lipodystrophy syndrome in HIV-infected children are limited but needed:
  - to understand factors associated with the emergence, persistence and progression or regression of symptoms
  - to elucidate associations between body fat alterations and metabolic abnormalities
  - to clarify the cumulative effect of HAART exposure over time on dyslipidemia
- European Paediatric Lipodystrophy Group published results from a cross-sectional study in 2004
- New European prospective study established to extend this previous work in centres participating in the ECS and the Italian Register of HIV infection in children
### Active Surveillance Study: Population

465 children and young adults were recruited

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age (yrs)</td>
<td>13.5 (IQR 9.9-17)</td>
</tr>
<tr>
<td>Male</td>
<td>49%</td>
</tr>
<tr>
<td>Black</td>
<td>22%</td>
</tr>
<tr>
<td>Vertically infected</td>
<td>93%</td>
</tr>
<tr>
<td>HCV coinfected</td>
<td>7%</td>
</tr>
<tr>
<td>Tanner stage I</td>
<td>28%</td>
</tr>
<tr>
<td>Tanner stage V</td>
<td>34%</td>
</tr>
<tr>
<td>Ever HAART use</td>
<td>95%</td>
</tr>
<tr>
<td>Median age of initiation (yrs)</td>
<td>3.6 (IQR 1.0-7.3)</td>
</tr>
<tr>
<td>Median total duration of ART (yrs)</td>
<td>8.7 (IQR 5.7-11)</td>
</tr>
<tr>
<td>Patients actually receiving PIs</td>
<td>55%</td>
</tr>
</tbody>
</table>
Prevalence of any-body fat changes and any-dyslipidemia

- Body fat redistribution: 43%
- Dyslipidemia: 27%
- Combined body fat redistribution and dyslipidemia: 14%
- No lipodystrophy: 44%

Frequency

- Body fat redistribution only: 29%
- Dyslipidemia only: 13%
- Combined body fat redistribution and dyslipidemia: 14%
- No lipodystrophy: 44%

Total: n = 208
Alterations in Body Fat

- Prevalence 6% to 56%—definition (clinical, skinfold, few DXA)
- Associated with duration of therapy, markers of disease severity, ART (d4T, PI)
- Lipoatrophy > lipohypertrophy
- Generally not very severe until puberty
- Female more than males
Atherosclerosis in children

Atherosclerosis begins in childhood and adolescence and progresses during young adulthood to cause cardiovascular disease in middle-aged and older individuals.

Autopsy studies of children and adolescents showed that the frequency and severity of atherosclerosis is associated with high LDL-cholesterol, hypertension, impaired glucose tolerance, obesity and low HDL-cholesterol.

Carotid intima-media thickness (IMT) measured by the Echo-Doppler technique is suitable an index of subclinical atherosclerosis.
HIV vertically-infected children provide the opportunity to investigate the impact of HIV infection and ART on vasculature without the confounding effect of the cumulative risk factors burden present by adulthood.

Cardiovascular disease is emerging as an important health concern at the third and fourth decades of life in HIV-infected patients.

It is critically important to determine the early impact of both the HIV infection and its treatment on the arterial wall in HIV-infected children.
## Cardiovascular diseases

### Endovascular studies

<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>Age range</th>
<th>Country</th>
<th>ARV</th>
<th>Control Group N matching</th>
<th>Major Finding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bonnet et al AIDS, 2004</td>
<td>49</td>
<td>3.5-19 yrs (mean: 13.5)</td>
<td>France</td>
<td>32 HAART 15 naive</td>
<td>N=24: age-sex</td>
<td>no differences in cIMT in HIV+: ↓ FMD</td>
</tr>
<tr>
<td>Charakida et al Circulation, 2005</td>
<td>83</td>
<td>5-17.7yrs (mean 11.0) 70% black</td>
<td>UK</td>
<td>31 PI 27 naive</td>
<td>N=59</td>
<td>in HIV+: ↑ cIMT ↓ FMD relevant factors: age, CDC stage, ARV and PI use</td>
</tr>
<tr>
<td>McComsey et al AIDS, 2005</td>
<td>31</td>
<td>2-20 yrs (mean: 9)</td>
<td>USA</td>
<td>16 PI 15 NNRTI</td>
<td>N=31 age-sex-race-BMI</td>
<td>Only duration of ART predictive of IMT: no association with traditional risk factors</td>
</tr>
<tr>
<td>Giuliano et al Coron Art Dis, 2008</td>
<td>83</td>
<td>(mean age 10 ±2.9)</td>
<td>Brazil</td>
<td>37 PI 6 No ARV</td>
<td>N=83 age-sex-economic class Race NA</td>
<td>in HIV+: ↑ cIMT;</td>
</tr>
</tbody>
</table>
Both HIV-Infection and Long-Term Antiretroviral Therapy are Associated with Increased Common Carotid Intima-Media Thickness in

Viganò et al. Curr HIV Res 2010

Background

• It is unclear whether HIV infection or ART or their combination is the responsible for an increased risk of atherosclerosis in HIV-infected children.

• Carotid intima-media thickness (IMT) is a suitable index of subclinical atherosclerosis.
Methods

Case-control study on 23 HIV-infected adolescents and young adults receiving ART (F 10; mean age 20 yrs; mean BMI 19.9) and 19 HC.

Cases and HC were Caucasian, matched by age (± 1 year), gender and BMI (± 1 kg/m2).

Cases and HC underwent clinical, anthropometric, and laboratory assessment, and CCIMT measurements by Echo-Doppler.
<table>
<thead>
<tr>
<th></th>
<th>HIV+ (n = 23)</th>
<th>HC (n = 19)</th>
<th>Unpaired t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>CD4+ cells (fluorescence units)</td>
<td>690</td>
<td>373</td>
<td>—</td>
</tr>
<tr>
<td>Total Cholesterol (mg / dL)</td>
<td>160</td>
<td>33</td>
<td>173</td>
</tr>
<tr>
<td>HDL-cholesterol (mg / dL)</td>
<td>53</td>
<td>15</td>
<td>66</td>
</tr>
<tr>
<td>LDL-cholesterol (mg / dL)</td>
<td>83</td>
<td>—</td>
<td>88</td>
</tr>
<tr>
<td>Triglycerides (mg / dL)</td>
<td>91</td>
<td>—</td>
<td>74</td>
</tr>
<tr>
<td><strong>Folate (ng / mL)</strong></td>
<td>4.7</td>
<td>1.5</td>
<td>6.9</td>
</tr>
<tr>
<td>Vitamin B12 (pg / mL)</td>
<td>481</td>
<td>—</td>
<td>463</td>
</tr>
<tr>
<td>Homocysteine (μmol / L)</td>
<td>11</td>
<td>—</td>
<td>9</td>
</tr>
<tr>
<td>Glucose (mg / dL)</td>
<td>80</td>
<td>8</td>
<td>81</td>
</tr>
<tr>
<td>Insulin (mg / dL)</td>
<td>7</td>
<td>—</td>
<td>6</td>
</tr>
<tr>
<td>HOMA-R</td>
<td>1.3</td>
<td>—</td>
<td>1.3</td>
</tr>
<tr>
<td><strong>CCIMT (mm)</strong></td>
<td>0.5</td>
<td>0.1</td>
<td>0.4</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>112</td>
<td>—</td>
<td>110</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>67</td>
<td>—</td>
<td>68</td>
</tr>
<tr>
<td>CD36+ cells (fluorescence units)</td>
<td>519</td>
<td>—</td>
<td>352</td>
</tr>
</tbody>
</table>
# Bootstrap selection of predictors

<table>
<thead>
<tr>
<th>Predictor</th>
<th>BIF (out of 1000)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV status</td>
<td>942</td>
</tr>
<tr>
<td>Gender</td>
<td>654</td>
</tr>
<tr>
<td>Mean blood pressure*</td>
<td>387</td>
</tr>
<tr>
<td>Triglycerides*</td>
<td>335</td>
</tr>
<tr>
<td>Homocysteine*</td>
<td>334</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>312</td>
</tr>
<tr>
<td>BMI</td>
<td>306</td>
</tr>
<tr>
<td>Waist Circumference</td>
<td>289</td>
</tr>
<tr>
<td>CD36+ cells*</td>
<td>287</td>
</tr>
<tr>
<td>Age</td>
<td>270</td>
</tr>
<tr>
<td>Folate</td>
<td>243</td>
</tr>
<tr>
<td>LDL*</td>
<td>238</td>
</tr>
<tr>
<td>HOMA-R*</td>
<td>202</td>
</tr>
</tbody>
</table>

*BIF= bootstrap inclusion fraction

* Selection on $\log_e$-transformed variable
Prediction of CCMT from HIV status and gender

<table>
<thead>
<tr>
<th>Regression coefficient or measure of model fit [bootstrapped 95%CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HIV</strong> (1 = yes; 0 = no)</td>
</tr>
<tr>
<td>0.13** [0.09 to 0.17]</td>
</tr>
<tr>
<td><strong>Male gender</strong> (1 = yes; 0 = no)</td>
</tr>
<tr>
<td>0.08** [0.03 to 0.12]</td>
</tr>
<tr>
<td><strong>Intercept</strong></td>
</tr>
<tr>
<td>0.36 [0.33 to 0.39]</td>
</tr>
<tr>
<td><strong>R^2_{adj}</strong></td>
</tr>
<tr>
<td>0.54** [0.36 to 0.73]</td>
</tr>
<tr>
<td><strong>RMSE (mm)</strong></td>
</tr>
<tr>
<td>0.07** [0.06 to 0.08]</td>
</tr>
</tbody>
</table>

* p < 0.05, ** p < 0.001

R2adj=adjusted coefficient of determination:
RMSE= room mean squared error
Jourdan C. et al recently published age-specific standard deviation scores (SDS) of CCIMT for Caucasians aged 10-20 years. When we applied these SDS to our subjects aged ≤ 20 years (n = 30), we found that the mean SDS correspond to the 98th percentile in HIV subjects and to the 46th percentile in controls.

This finding confirms that the difference between HIV and controls reported in our study is clinically meaningful.

PATIENTS’ ART HISTORY

**At the beginning of ART**
- ZDV / ZDV+ddI

**At enrollment**
- NNRTI-based regimen

**Previuos regimen**
1. Naive to ART
2. Two PIs-based regimens*
3. Single PI-based regimen*
4. Three PIs-based regimens*
5. Single RTV-boosted PI-based regimen

**HIV-infected patients**
- 23

* Unboosted with RTV
Significant (p= 0.019) association between CCIMT and the duration of ART in subjects exposed to a PI-based and/or NNRTI-based regimen plus a single or double NRTIs.

This association was no longer present after consideration of the duration of PI- and/or NNRTI-based regimen, NNRTI-based regimen or PI-based regimen.
CONCLUSIONS

Our case-control study of HIV-infected adolescents and young adults shows that:

CCIMT was higher in cases as compared to HC of the same gender, age and BMI

HIV infection, male gender and long duration of ART are risk factors for higher CCIMT
A dynamic organ, bone is continuously (re)modelled

“coupling”
Bone formation
- Osteoblast
- Osteoid
- Bone matrix

Bone resorption
- Osteoclast
- Periosteal surface
- Bone formation

Markers:
- Alkaline phosphatase (ALP) (serum)
- Osteocalcin (OC) (serum)
- Bone-specific alkaline phosphatase (BALP) (serum)
- Propeptide of type I procollagen (PINP, PICP) (serum)
- Pyridinoline (PYR)
- Deoxypyridinoline (DPD) (urine, serum)
- Hydroxyproline (OHP) (urine)
- Cathepsin K (serum)
- Tartrate-resistant acid phosphatase (TRAP) (serum)
- Calcium (urine)
- Telopeptide of type I collagen (NTx, CTX) (urine, serum)
Association Between Bone Density and Fractures in Children: A Systematic Review and Meta-analysis

Old bones in young bodies?

Genes, sex, hormones, nutrition, exercise, sex-steroids, GH-IGF-I axis; adipose and muscle tissue Ca++, Vit D

Skeletal bone mass doubles between onset of puberty and young adulthood

Accelerated loss during menopause then gradual loss in elderly

Peak bone mass

Cessation of growth

Bone Mass

Age (Years)
Old bones in young bodies?
Osteoclastogenesis

- RANK-L
- RANK
- Stromal Cell
- Osteoblast
- Osteoclast progenitors
- IL-1
- IL-17
- TNF-α
- PTH
- Glucocorticoids
- Differentiation, fusion, increased survival
- Osteoclast
- T Cell

Presented at the 2nd International Workshop on HIV Pediatrics
16-17 July 2010, Vienna Austria
Pathogenetic hypotheses

HIV

Activated T Cell

PI

Osteoblasts

Osteoclast precursor

RANKL

Osteoclast
Bone Density in HAART-treated Children and Youths

Significantly lower compared to healthy children (P < 0.0006)

Mora S et al. AIDS, 15:1823-1829; 2001
Bone Mineral Content in HIV-Infected Untreated Children

Mora S et al. Calcif Tissue Int, 76:336-40; 2005
Bone Metabolism in HIV-Infected Children and Youths (I)

Mora S et al. AIDS, 15:1823-1829; 2001

35 HAART treated children and adolescents
314 Healthy controls

Significantly higher compared to healthy children (P < 0.0008)
Bone Metabolism in HIV-Infected Children and Youths (II)

27 HAART treated children and adolescents
336 Healthy controls

![Graphs showing significantly higher levels of BALP and NTx in HIV-infected children compared to healthy controls.](image)

*Significantly higher compared to healthy children (P < 0.001)*

RANKL and OPG

* Significantly higher compared to healthy children (P < 0.0001)

RANKL/OPG ratio

* Significantly higher compared to healthy children (P = 0.02)

The available data indicate that bone metabolism derangement is present in children, adolescents and young adults taking HAART, and that such alterations might be the cause of the detected low BMD values.

Mora S. & Viganò A.  
*Frontiers in Bioscience, (in press)*
Presented at the 2nd International Workshop on HIV Pediatrics
16-17 July 2010, Vienna Austria

Antiretroviral Therapy and Bone Measurements in HIV-Infected Youths

Aim: to assess the effect of different antiretroviral drugs on bone mass in HIV-infected youths

Zuccotti GV et al, Bone. 2010
Presented at the 2nd International Workshop on HIV Pediatrics

16-17 July 2010, Vienna Austria

Subjects

82 Patients

Highly active antiretroviral therapy (HAART) containing a protease inhibitor (PI)

HAART containing a non-nucleoside reverse transcriptase inhibitor (NNRTI)

Dual nucleoside reverse transcriptase inhibitor (NRTI)
<table>
<thead>
<tr>
<th>Subjects</th>
<th>ARV naïve</th>
<th>Dual NRTI</th>
<th>PI-based HAART</th>
<th>NNRTI-based HAART</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>10 Girls/5 Boys</td>
<td>7 Girls/4 Boys</td>
<td>17 Girls/15 Boys</td>
<td>13 Girls/15 Boys</td>
</tr>
<tr>
<td>Age (y)</td>
<td>11.6 (1.3)</td>
<td>11.5 (1.5)</td>
<td>14.7 (0.5)</td>
<td>15.7 (0.7)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>36.9 (4.2)</td>
<td>43.5 (6.3)</td>
<td>49.1 (2.4)</td>
<td>51.0 (2.3)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>139.3 (6.3)</td>
<td>140.9 (7.9)</td>
<td>154 (2.5)</td>
<td>158.7 (2.1)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>17.9 (0.7)</td>
<td>20.4 (1.2)</td>
<td>20.2 (0.5)</td>
<td>20.0 (0.5)</td>
</tr>
<tr>
<td>CD4 (n)</td>
<td>279 (15-902)</td>
<td>733 (359-1305)</td>
<td>840 (289-1798)</td>
<td>814 (283-38647)</td>
</tr>
<tr>
<td>CD4 (%)</td>
<td>15.7 (2.1-35.5)</td>
<td>24.5 (16.9-45.0)</td>
<td>29.9 (15.3-49.4)</td>
<td>35.8 (23.3-49.8)</td>
</tr>
<tr>
<td>HIV-RNA</td>
<td>20913 (266-500000)</td>
<td>1666 (49-95652)</td>
<td>49 (49-1760)</td>
<td>49 (49-37317)</td>
</tr>
<tr>
<td>ARV duration (mo)</td>
<td>55.6 (6.6)</td>
<td>45.2 (4.8)</td>
<td>41.9 (3.1)</td>
<td></td>
</tr>
</tbody>
</table>
Subjects

194 Controls

Age: 4.9 - 21.9 y
Weight: 17.5 - 100 kg
Height: 109 - 186 cm
BMI: 12.4 - 29.3 kg/m²

Healthy children and adolescents.
None had a history of endocrine, nutritional, growth or renal problems.
Results (I)
Results (II)

IDV: indinavir; NFV: nelfinavir; RTV: ritonavir; LPV/r: lopinavir/ritonavir

* Significantly different from others (P < 0.001)
Role of stavudine (d4T)


A significant loss of bone mineral density was observed only in patients receiving the standard dose of Stavudine (d4T), but not in patients receiving half the dose of the drug.
Results (IV)

A = healthy controls
B = ARV naïve
C = dual NRTI + d4T
D = dual NRTI no d4T
E = d4T + PI no RTV full dose
F = d4T+ RTV full dose
G = NRTI different from d4T + PI no RTV
H = TDF plus 3TC plus EFV
Conclusions

- Our data indicate that ARV may have a detrimental effect on bone health of HIV-infected children and adolescents.

- The use of **full dose RTV** or the combination of **d4T plus RTV (full dose)** are associated to lower bone mass measurements, and therefore should be monitored closely.
Summary

- ARV saves lives!

- Prolonged ARV exposure may place HIV-infected children at very high risk for:
  - Metabolic and morphologic abnormalities, premature atherosclerosis and increased risk of fracture.

- We urgently need data to inform monitoring and treatment.