Advances in the Microbiological Diagnostic of Opportunistic Infections in AIDS Patients

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OUTLINE

1. Incidence of OIs in c-ART era
2. Why OIs still happening?
3. *Pneumocystis* pneumonia
4. Tuberculosis
5. Toxoplastic encephalitis
6. Geographical OIs
7. Conclusions
Annual Incidence of first AIDS-defining OIs, 1994-2007

HIV-Associated Opportunistic Infections-Going, Going, But Not Gone.

“OIs are here to stay…”

Opportunistic Infection

Host-Parasite Interaction

WHY OIs STILL HAPPENING?

• OIs as HIV Debut

• Patients w/o Prophylaxis /Treatment

• Treatment Failure

• Failure to Reinitiate Prophylaxis (↓ CD₄)
PATHOGENESIS OF OIs IN AIDS PATIENTS

- Reactivation of latent infection
  - M. tuberculosis
  - P. jirovecii*, T. gondii
  - Virus (CMV, VZV, HSV, JC, EBV)
  - Other microorganisms

- Saprophitic microorganisms proliferation
  - Candida albicans

- Exogenous microorganisms
  - Coccidia
  - Cryptococcus sp
  - Other microorganisms
AIDS-INDICATED DISEASES FREQUENCY
CorIS (N=477; 2004-07)

- OI: 79%
- KS: 8%
- NHL: 5%
- Cervix: 6%
- ADC: 1%
- WS: 1%
AIDS-INDICATED DISEASES ACCORDING TO TRANSMISSION RISK FACTORS
CoRIS (N= 477; 2004-07)
OI's AT AIDS DEBUT
CoRIS (N= 477; 2004-07)
OIs FREQUENCY ACCORDING TO TRANSMISSION RISK FACTORS
CoRIS (N= 477; 2004-07)
Place of birth

SPAIN 71.5%

Migrants 28.5%

- Latinoamérica y Caribe
- África subsahariana
- Europa Occidental
- E Este y Rusia
- Norte de África
- Otros/NC

- 60
- 34
- 93
- 42
- 145
- 400
<table>
<thead>
<tr>
<th>Diseases</th>
<th>Men</th>
<th></th>
<th>Women</th>
<th></th>
<th>Total</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
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<tr>
<td><strong>EU/EEA</strong></td>
<td></td>
<td></td>
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<tr>
<td>Pneumocystis pneumonia</td>
<td>842</td>
<td>24.6</td>
<td>256</td>
<td>23.9</td>
<td>1127</td>
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<td>Candidiasis, oesophageal</td>
<td>428</td>
<td>12.5</td>
<td>107</td>
<td>13.1</td>
<td>535</td>
<td>12.7</td>
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<td>Mycobacterium tuberculosis</td>
<td>369</td>
<td>10.9</td>
<td>157</td>
<td>13.1</td>
<td>526</td>
<td>11.4</td>
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<tr>
<td>Mycobacterium tuberculosis, extrapulmonary</td>
<td>298</td>
<td>8.7</td>
<td>128</td>
<td>10.7</td>
<td>426</td>
<td>9.2</td>
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<tr>
<td>Kaposi's sarcoma</td>
<td>334</td>
<td>9.8</td>
<td>36</td>
<td>3.0</td>
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<td>Wasting syndrome due to HIV</td>
<td>272</td>
<td>8.0</td>
<td>81</td>
<td>6.8</td>
<td>353</td>
<td>7.7</td>
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<tr>
<td>Toxoplasmosis of brain</td>
<td>218</td>
<td>6.4</td>
<td>110</td>
<td>9.2</td>
<td>328</td>
<td>7.1</td>
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<tr>
<td>CMV disease (other than liver, spleen, or nodes)</td>
<td>142</td>
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<td>60</td>
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<td>206</td>
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<tr>
<td>Encephalopathy, HIV-related</td>
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<td>Progressive multifocal leukoencephalopathy</td>
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<td>32</td>
<td>2.7</td>
<td>140</td>
<td>3.0</td>
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<tr>
<td>Opportunistic infection(s), not specified</td>
<td>242</td>
<td>6.4</td>
<td>49</td>
<td>4.1</td>
<td>291</td>
<td>5.8</td>
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<tr>
<td><strong>Non-EU/EEA</strong></td>
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<tr>
<td>Mycobacterium tuberculosis</td>
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<td>643</td>
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<tr>
<td>Candidiasis, oesophageal</td>
<td>283</td>
<td>21.7</td>
<td>166</td>
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<td>24.0</td>
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<tr>
<td>Wasting syndrome due to HIV</td>
<td>208</td>
<td>16.0</td>
<td>91</td>
<td>16.0</td>
<td>299</td>
<td>16.0</td>
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<tr>
<td>Mycobacterium tuberculosis, extrapulmonary</td>
<td>96</td>
<td>7.4</td>
<td>30</td>
<td>5.3</td>
<td>126</td>
<td>6.7</td>
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<tr>
<td>Pneumocystis pneumonia</td>
<td>81</td>
<td>6.2</td>
<td>29</td>
<td>5.1</td>
<td>110</td>
<td>5.9</td>
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<tr>
<td>Encephalopathy, HIV-related</td>
<td>57</td>
<td>4.4</td>
<td>29</td>
<td>5.1</td>
<td>86</td>
<td>4.6</td>
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<tr>
<td>Candidiasis of bronchi, trachea, or lungs</td>
<td>41</td>
<td>3.1</td>
<td>18</td>
<td>3.2</td>
<td>59</td>
<td>3.2</td>
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<tr>
<td>Toxoplasmosis of brain</td>
<td>28</td>
<td>2.1</td>
<td>21</td>
<td>3.7</td>
<td>49</td>
<td>2.6</td>
</tr>
<tr>
<td>Pneumonia, recurrent in an adult or an adolescent</td>
<td>33</td>
<td>2.5</td>
<td>14</td>
<td>2.5</td>
<td>47</td>
<td>2.5</td>
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<tr>
<td>Mycobacterium, other species or unidentified species</td>
<td>26</td>
<td>2.0</td>
<td>15</td>
<td>2.6</td>
<td>41</td>
<td>2.2</td>
</tr>
<tr>
<td>Opportunistic infection(s), not specified</td>
<td>259</td>
<td>19.9</td>
<td>159</td>
<td>27.9</td>
<td>418</td>
<td>22.3</td>
</tr>
</tbody>
</table>
Pneumocystis jirovecii
PNEUMONIA (PcP)
Incidence of PcP in HIV-infected Patients
Hospital Clínic Barcelona 1984 -2008

HAART: Highly Active Antiretroviral Therapy (≥2NRTI plus ≥1PI/NNRTI)

PcP PROPHYLAXIS

• PcP debut of HIV
• Patients without prophylaxis/ treatment
• Treatment failure
PcP DIAGNOSIS

- **Type of Sample**
  - BAL, IS, pulmonar and transbronchial biopsy

- **Optical microscopy**
  - Stains:
    - Silver Methenamine Gomori
    - Toluidine blue,
    - Giemsa, Gram, Papanicolau
  - Immunofluorescence

- **Electronic microscopy**

- **Molecular biology techniques**
  - > Sensitivity & Specificity
  - Non invasive samples: OW
  - Asymptomatic carriers diagnostic

- **Other techniques:** plasma (S-adenosilmetionina)
  - serum (β-glucans)
BAL Silver Methenamine stained 100X

(magnified)

Courtesy Dr. J Mas
Sensitivity and specificity of nested and real-time PCR for the detection of *Pneumocystis jiroveci* in clinical specimen

Míriam J. Alvarez-Martínez, José M. Miró, Maria Eugenia Valls, Asunción Moreno, Paula V. Rivas, Manel Solé, Natividad Benito, Pere Domingo, Carmen Muñoz, Esteban Rivera, Heather J. Zar, Gustavo Wissmann, Ada R.S. Diehl, João C. Prolla, Maria Teresa Jiménez de Anta, José M. Gatell, Paul E. Wilson, Steven R. Meshnick, and the Spanish PCP Working Group

Diagnostic Microbiology and Infectious Disease 56 (2006) 153–160

**Quantitative rT-PCR**
Detection of *P. jiroveci* by nested PCR and real time PCR in positive and negative specimens

<table>
<thead>
<tr>
<th>Microscopy</th>
<th>No.</th>
<th>Real-time PCR, ( n (%) )</th>
<th>Nested PCR, ( n (%) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive specimens</td>
<td>71</td>
<td>67 (94)</td>
<td>67 (94)</td>
</tr>
<tr>
<td>Negative specimens</td>
<td>70</td>
<td>3 (4)</td>
<td>13 (19)</td>
</tr>
</tbody>
</table>

Quantitative rT-PCR showed statistically better specificity \((p=0.015)\) than nested-PCR, reducing false positive percentage.
1. Increased **risk of mortality** in following three months after diagnosis (*Helweng-Larsen et al.*)

2. Sulfa-drugs prophylaxis is associated to presence of DHPS mutations but **outcome is not affected** (*Kazanjian & Meshnick*)

3. Sulfa-drugs prophylaxis is an **independent factor** for DHPS mutations (*Navin et al.*)
**Pneumocystis jirovecii** pneumonia in Spanish HIV infected patients in the combined antiretroviral therapy era: prevalence of dihydropteroate synthase mutations and prognostic factors of mortality.

Míriam J. Alvarez-Martínez, Asunción Moreno, José M. Miro, Maria Eugenia Valls, Paula V. Rivas, Elisa de Lazzari, Omar Sued, Natividad Benito, Pere Domingo, Esteban Ribera, Miguel Santín, Guillermo Sirera, Ferrán Segura, Francesc Vidal, Francisco Rodríguez, Melchor Riera, Maria Elisa Cordero, José Ramón Arribas, María Teresa Jiménez de Anta, José M. Gatell, Paul F. Wilson, Steven R. Meshnick.

Spanish PCP Working Group.

Diagnostic Microbiology and Infectious Disease 62 (2008) 34–43

- **Prevalence of** *Pneumocystis* DHPS gene in Spain in c-ART era: **3.7%**
- **Global mortality** 15%, rising to 80% in patients with mechanical ventilation.
- Presence of DHPS mutations were not associated to worse outcome.
- **TMP-SMX is effective at therapeutica doses.**
• DHPS mutations more frequent in pre-cART (1989-1995) era, than in c-ART era (2001-2004), 33% vs. 5.5%, respectively.

- Sulfa –drugs prophylaxis
- Pre c-ART period
- HMS

Increase risk of mutations
Future:
Non-invasive Test for PcP Diagnosis

• Oral or oropharyngeal wash specimens + PCR assays.
  – S: up to 88%; Sp: up to 90%

• Plasma S-adenosylmethionine (SAM) ↓
  – Depletion (Skelly et al., CID, 2008)

• Serum (1-3)-beta- D- glucan ↑
  – Variable S & Sp

Efficiency of Oral Washes & BAL in PcP Diagnosis by real-time PCR  
Míriam J. Álvarez-Martínez et al. SEIMC-20

<table>
<thead>
<tr>
<th></th>
<th>BAL</th>
<th>OW</th>
</tr>
</thead>
<tbody>
<tr>
<td>sensitivity(S)</td>
<td>100%</td>
<td>53%</td>
</tr>
<tr>
<td>especificity (E)</td>
<td>78%</td>
<td>100%</td>
</tr>
<tr>
<td>positive predictive value (PPV)</td>
<td>88%</td>
<td>100%</td>
</tr>
<tr>
<td>negative predictive value (NPV)</td>
<td>100%</td>
<td>56%</td>
</tr>
</tbody>
</table>

**rT-PCR in BAL**
- a) Excelent sensitivity according to microscopy.
- b) PcP diagnosis in cases of low number of cyst ndetectable by microscopy.
- c) Allows *Pneumocystis* quantification.

**rT-PCR in Oral Washes**
- a) Very good specificity, further research is needed to improve sensitivity and consider it as an alternative diagnosistic method.
TUBERCULOSIS
MICROBIOLOGICAL DIAGNOSIS

Based on

1. Bacilloscopy
2. Culture
3. DNA Detection of *M. tuberculosis*
SPECIFIC STAINS

Ziehl-Neelsen

AURAMINA

RED ROADS, BLUE BACKGROUND

FLUORESCENCE ROADS, DARK BACKGROUND
GENETIC AMPLIFICATION

*M. Tuberculosis* ideal microorganism for genetics

- Slow growth
- Pathogenicity
- Complex conventional techniques

Different methods and techniques described
16S rRNA Gene Sequence

16s gene has conserved areas common to all bacteria and specie-specific areas (hipervariable areas)

Amplification of hipervariable areas from the conserved area and comparison to consensus sequence, identifies species.

Conserved area  Hipervariable area  Conserved area
REAL TIME PCR (RT-PCR)
GENETIC AMPLIFICATION

SENSITIVITY

Lower than culture
Positive culture + positive ZN: 95-100%
Positive culture + negative ZN: 60-65%

Low sensitivity & some false positive results

Low PPV_ Indicated if moderated-high TB suspicion
GENETIC AMPLIFICATION

SENSITIVITY

It has not reached all expectations

Difficulties in bacteria lysis & DNA extraction

Consensus:
- Use of commercial automatized systems
- Detection of drugs resistances
Diagnosis of TB Resistance

Slow classical systems

MOLECULAR BIOLOGY CHALLENGE

Molecular basis of resistance

Chromosomal mutations in known genes

Not in all cases of resistances mutations are found
Hybridisation of wild type strain

Hybridisation of mutant strain showing resistance
Diagnosis of Resistance

Genotype® MTB DR Plus
(Solid-Phase Hybridization assay)
GenoType Mycobacterium Direct Assay

PCR amplification and hybridization with the probe attached to the strip.
Diagnosis of Resistance

GeneXpert® MTB/RIF

Detection of resistance to RF in bacilloscopy- positive clinic samples

Closed system (DNA extraction, amplification and detection)
**Figure 2. Assay Procedure for the MTB/RIF Test.**
Two volumes of sample treatment reagent are added to each volume of sputum. The mixture is shaken, incubated at room temperature for 15 minutes, and shaken again. Next, a sample of 2 to 3 ml is transferred to the test cartridge, which is then loaded into the instrument. All subsequent steps occur automatically. The user is provided with a printable test result, such as "MTB detected; RIF resistance not detected." PCR denotes polymerase chain reaction.
IGRA’s

- **Interferon-γ-release assays**
- **Alternative to tuberculin skin test (TST)**
  - QuantiFERON-TB Gold Intube (QFT-IT)
    - Whole blood assay
    - S: 64% in HIV-infected adults; 79% in HIV-negative
  - **TB-ELISPOT**
    - Peripheral blood mononuclear cells (PBMCs)
    - Overall sensitivity higher
    - Scarce data comparing HIV-pos/HIV-neg
    - More accuracy, promising in other samples than blood
Figure 1. Comparison of T-SPOT.TB and Quantiferon-TB Gold In Tube (QFT-IT) methodology. ELISA: enzyme-linked immunosorbent assay; ELISPOT: enzyme-linked immunospot assay; IFN: interferon; PBMCs: peripheral blood mononuclear cells.
Toxoplasmic Encephalitis
Diagnostic Approach of Focal Brain Lesions

Contrast-enhancing mass lesion/s (CT/MRI)

YES

\[ ^{201}\text{TI SPECT/PET} \]

+ 

PCNSL

- 

Toxoplasma serology

+ 

CSF sampling when feasible !!!

- 

TE

- PML

- Other

- Tuberculoma

- Cryptococcoma

- Other
Molecular Diagnosis: PCR

- Avoids invasive samples (biopsy)
- Useful samples in immunosuppressed patients
  - BAL
  - CFS
  - Vitreous & aqueous humor
  - Pleural & peritoneal liquid
  - Bone marrow
  - Peripheral blood

- Lack of standarised protocols
- Nested PCR, heminested PCR, rT-PCR
- Genes
  - B1; AF146527; P30; rRNA; α-tubuline, β-tubuline; TGR1E
PCR: Indications.
Sensitivity & Specificity

**Indications**

- Confirm clinical, radiological & serological in immunosuppressed patients.
- Follow up of infected patients and on risk non-infected patients.

**S & Sp**

- PCR in blood (Encephalitis)
  - S (15-85%), less if previous treatment
  - Sp: 100%
- PCR vitreous (Ocular)
- PCR in BAL (Pulmonar)
  - S, Sp: 100%
Design of a one-tube hemi-nested PCR for detection of *Toxoplasma gondii* and comparison of three DNA purification methods

MARC PUJOL-RIQUÉ, FRANCIS DEROUIN*, ALBERTO GARCÍA-QUINTANILLA, M. E. VALLS, J. M. MIRÓ† and M. T. JIMÉNEZ DE ANTA

- B1-gene target
- S: 87.5%

JMM, 1999.
Evaluation of a new 5′-nuclease real-time PCR assay targeting the *Toxoplasma gondii* AF146527 genomic repeat

J. Menotti¹, Y. J-F. Garin¹, P. Thulliez⁴, M-C. Sérugue¹, J. Stanislawski², P. Ribaud², N. de Castro³, S. Houzé⁵ and F. Derouin¹

**TABLE 1.** Results of prospective assessment of the PCR–ELISA assay that targets the *Bl* gene and the TaqMan-based real-time PCR assay that targets the AF146527 DNA sequence

<table>
<thead>
<tr>
<th>Patient</th>
<th>Clinical background</th>
<th>Specimen</th>
<th>Date</th>
<th>PCR–ELISA</th>
<th>Real-time PCR (no. tachyzoites/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>HIV infection</td>
<td>CSF</td>
<td>11 October</td>
<td>+</td>
<td>3600</td>
</tr>
<tr>
<td>P2</td>
<td>HIV infection</td>
<td>CSF</td>
<td>15 October</td>
<td>+</td>
<td>71</td>
</tr>
<tr>
<td>P3</td>
<td>HIV infection</td>
<td>CSF</td>
<td>22 November</td>
<td>+</td>
<td>67</td>
</tr>
<tr>
<td>P4</td>
<td>HIV infection</td>
<td>Whole blood</td>
<td>4 December</td>
<td>+</td>
<td>7.9</td>
</tr>
<tr>
<td>P5</td>
<td>HIV infection</td>
<td>Whole blood</td>
<td>6 December</td>
<td>+</td>
<td>180</td>
</tr>
<tr>
<td>P6</td>
<td>Toxoplastic seroconversion during pregnancy</td>
<td>Blood buffy coat</td>
<td>11 December</td>
<td>+</td>
<td>6200</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bone marrow</td>
<td>13 December</td>
<td>+</td>
<td>3900</td>
</tr>
<tr>
<td>P7–P61</td>
<td>Various</td>
<td>Various</td>
<td>13 December</td>
<td>+</td>
<td>16 000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Various</td>
<td>21 December</td>
<td>–</td>
<td>3.3</td>
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<tr>
<td></td>
<td></td>
<td>Various</td>
<td>23 December</td>
<td>–</td>
<td>140</td>
</tr>
</tbody>
</table>

+ presence of *T. gondii* DNA detected by PCR–ELISA; –, PCR–ELISA negative.

*Allogeneic stem cell transplantation, HIV infection, renal transplantation, pulmonary transplantation, idiopathic lymphopenia, toxoplastic seroconversion during pregnancy.

bWhole blood, blood buffy coat, serum, cerebrospinal fluid (CSF), brain biopsy, bronchoalveolar lavage (BAL) fluid, aqueous humour, placenta.
GEOGRAPHIC OI’S

• Chagas Disease (*T. cruzi* infection)
• Visceral leishmaniasis
• Fungal infections
  – *Histoplasma capsulatum*
  – *Penicillium*
• *Strongyloides stercoralis*
CONCLUSIONS

• OIs has been decreasing dramatically after c-ART.
• However,... Ols still here.
• Classical diagnostic techniques are in use (microscopy/serology & culture)
• New molecular methods complement classical diagnosis:
  - advantages: rapidity; quantification; monitor treatment response; study of resistances; molecular epidemiology.
  - disadvantages: expensive; equipment.
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- Dr. José María MIRÓ
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- Dr. María Eugenia Valls
- Dr. Jordi Mas
  - Microbiology, Hospital Clinic, Barcelona
- Dr. Steven Meshnick
  - University North Carolina, Chapel Hill, NC, USA.
You are invited to join us in Barcelona and to learn much more about

\textit{Pneumocystis}\textit{ Toxoplasma}\textit{ Trypanosoma cruzi}\textit{ Leishmania}