4th International HIV/Viral Hepatitis Co-Infection Meeting

The Rocky Road to Viral Hepatitis Elimination:
Assuring access to antiviral therapy for ALL co-infected patients from low to high income settings

Saturday - Sunday, 22-23 July 2017
Paris, France
The evolution in HBV markers

Slim Fourati
« Classical » HBV markers

- HBsAg/anti HBs Ab
- Anti HBc Ab
- HBeAg/anti HBe Ab
- HBV DNA level
- HBV genotype
  Pre C and BCP variants
- ALT level
- Fibrosis status

Diagnosis

Define the phases

Prognosis (cirrhosis, HCC)

Treatment indication
Need for new biomarkers

- To better define the phases of infection
- To define the ideal duration of treatment
- To monitor antiviral treatment efficacy
- To monitor the efficacy of novel strategies aiming at viral cure
Part I
Optimize screening
Rapid diagnostic tests for HBsAg
Rapid diagnostic tests (RDTs) for HBsAg

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Alere Determine™ HBsAg</th>
<th>VIKIA® HBsAg</th>
<th>DRW HBsAg rapid Test</th>
<th>Toyo HBsAg Rapid Test</th>
<th>Assure® HBsAg rapid test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manufacturer</td>
<td>Alere USA</td>
<td>bioMérieux France</td>
<td>DRW Ltd. USA</td>
<td>Turklab Turkey</td>
<td>MP Biomedicals Singapore</td>
</tr>
<tr>
<td>Specimen type</td>
<td>Whole blood, serum, plasma</td>
<td>Whole blood, serum, plasma</td>
<td>Serum, plasma</td>
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</tr>
<tr>
<td>Volume needed (µL)</td>
<td>50</td>
<td>75</td>
<td>80</td>
<td>100</td>
<td>50, 75 (S,P)</td>
</tr>
<tr>
<td>Time required (min)</td>
<td>15</td>
<td>5-15</td>
<td>30</td>
<td>5-15</td>
<td>15-20</td>
</tr>
</tbody>
</table>

*Courtesy of Stéphane Chevaliez*
Performances of RDTs- HBsAg (whool blood)

- **2472-3928 tested individuals**
- **High rates of successful results** (Determine™: 100%; Vikia®: 99,8%; Quick Profile™: 98,1%)

<table>
<thead>
<tr>
<th>Tests</th>
<th>Specificity</th>
<th>Sensitivity</th>
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<tbody>
<tr>
<td>Determine™</td>
<td>100%</td>
<td>93,6%</td>
</tr>
<tr>
<td>VIKIA®</td>
<td>99,9%</td>
<td>96,5%</td>
</tr>
<tr>
<td>Quick Profile™</td>
<td>99,7%</td>
<td>90,5%</td>
</tr>
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</table>

False negatives were typically observed in inactive carriers.
HBsAg POC tests (whool blood) in an african community setting

- 1000 subjects from 3 studies within the PROLIFICA program in the Gambia (field conditions).

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<tr>
<td>Determine™</td>
<td>100%</td>
<td>88.5%</td>
</tr>
<tr>
<td>VIKIA®</td>
<td>99.8%</td>
<td>90.0%</td>
</tr>
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</table>

Most of the patients with false-negative results were classified as inactive chronic carriers.
Rapid diagnostic tests (RDTs) for HBsAg (whole blood)

- 272 HIV-1 infected ARV naïve patients (rural Tanzania)
  - 9.2% HBsAg (+)

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<td>Determine™</td>
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<td>96.0%</td>
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There is a degree of uncertainty about the estimates due to the limited sample size.

Previous evaluation of Determine HBsAg in HIV patients has shown considerable heterogeneity of sensitivity between studies.

Part II
New Serum Biomarkers

Quantitative HBsAg
HBcrAg
HBV RNA
NEW BIOMARKERS?
HBV circulating particles and secreted proteins

Blood

HBeAg  HBsAg  Empty particles  « Immature » RNA particles  complete particles

Hepatocyte

Cytoplasm

Nucleus

Integration of Double-stranded linear HBV DNA

PgRNA packaging

cccDNA

dsDNA

Q-HBsAg: All forms of circulating HBsAg

HBeAg

HBsAg

Empty particles

RNA particles

Dane particles

Blood

Hepatocyte

Cytoplasm

Integration of Double-stranded linear HBV DNA

cccDNA

Hepatocyte

HBsAg quantification assays

- Three commercial assays quantify all forms of circulating HBsAg:
  - ARCHITECT HBsAg (Abbott)
  - Elecsys HBsAg II (Roche Diagnostics)
  - Liaison XL (Diasorin)

_reproducibility, automated quantification, standardization (IU/mL)_
Performance of HBsAg quantification assays

Sonneveld et al., J Clin Virol 2011 Jul;51(3):175-8
HBV core-related antigen (HBcrAg)

HBV core-related antigen (HBcrAg)

Denaturation and inactivation of antibodies

common linear epitope (149AA) detected by HBcrAg assay
HBV core-related antigen (HBcrAg) assay

- Only one commercially available immunoassay (Lumipulse G HBcrAg, Fujirebio).

- Requires detergent pre-treatment (denaturation)

- Volume of serum = 150 µL
Correlations of serum HBcrAg with intrahepatic cccDNA

Antiviral naïve patients

Reductions of HBcrAg paralleled with cccDNA reduction after 24M ETV
HBV RNA

Pre-genomic and spliced HBV RNA variants

Blood

Hepatocyte

Integration of Double-stranded linear HBV DNA

cccDNA

RNA particles

PgRNA

dsDNA

Dane particles

Correlations of serum HBV RNA with intrahepatic cccDNA

Treatment naïve HBeAg (+) CHB

Treatment naïve HBeAg (-) CHB
Potential Applications of biomarkers

- **Diagnosing active hepatitis vs inactive carrier**
  - **Quantitative HbsAg** (Brunetto et al. Gastroenterology 2010)
  - **HBcrAg** (Seto WK et al. Clin Microbiol Infect 2014; Maasoumy et al. Clin Microbiol Infect 2016)
  - **HBV RNA** (van Bommel et al. AASLD 2016)
Potential Applications of biomarkers

• **Predicting the response to treatment**
  
  – **PegIFNα** (quantitative HBsAg, HBcrAg, HBV RNA)

  – **Nucleos(t)ides analogs** (HBV RNA?)
HBsAg level is a treatment predictor to establish stopping rules for poor responders to PegIFNα

HBeAg (+)

Stopping rules at W12

Low probability of response
- W12: absence of decline from baseline (Gt- A and D)
- W12: HBsAg >20,000 IU/mL (GT-B and C)

High probability of response
W12: HBsAg< 1500 IU/mL (PPV =50%)

Response = HBeAg loss with HBV DNA <2,000 IU/mL at 6M-FU.
Slow HBsAg decline during NA therapy predicts low rates of HBsAg loss

Based on HBsAg kinetics, estimation of the median time to HBsAg loss in NA treated patients was 52.2 years (interquartile range: 30.8-142.7 years)

Chevaliez S et al. J hepatol 2013
Serum HBV RNA Levels predict HBeAg Seroconversion During Treatment With NAs

Serial serum samples from 62 CHB patients (50 HBeAg+) treated with LMV or TDF
Many validated HBsAg RDTs are reliable and now represent promising tools for large-scale screening and diagnosis of HBV infection.

New HBV biomarkers (e.g. HBcrAg and HBV RNA) may be satisfactory surrogate markers to intrahepatic HBV cccDNA.

These biomarkers could be used to:
- help distinguish active hepatitis versus inactive carrier status
- monitor NA or PegIFN based treatment and predict therapeutic efficacy

Further studies are needed to assess whether there is superiority of these markers for clinical decision making over established HBV biomarkers (e.g. HBsAg and HBV DNA quantification).