Hepatitis B Virus Serum Biomarkers
Virtual Workshop
October 5 & 12, 2020 GMT

Summary of Immunological Serum Biomarkers: Landscape
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12:25-12:45pm GMT, Oct 5th
Overview of the Presentation

1. Background in HBV immune pathogenesis

2. Immunological serum biomarkers associated with disease pathogenesis, clinical course (e.g. flares) and treatment outcomes

3. Summary and future directions
Background: HBV immune pathogenesis

- HBV is non-cytopathic
- Host immune response mediates both virus control and liver disease
- HBV-specific CD8 T-cells are the primary effectors that kill and cure HBV-infected hepatocytes
Background: HBV persists with global and virus-specific adaptive immune dysregulation

Adaptive Immune Dysregulation

↑ immune regulation:
- PD-1/PDL1, CTLA-4, Bim...
- Regulatory T-cells, cytokines and chemokines
- MDSC → Arginase
- Metabolic deficit
- HBV Ag

???With blunted adaptive immune responses, what causes the dynamic changes in:
- ALT
- HBV DNA
- HBeAg, HBsAg
- Disease progression

Likhitsup & Lok, Clin Liver Dis 23 (2019)
Background: HBV persists with differential immune activation and regulation in the liver

Innate cellular activation with inflammatory and/or regulatory effects:
- Kupffer cells, DC, NK, γδT, platelets
- MDSC, Tregs...
- LSEC, HSC

Soluble inflammatory and regulatory factors:
- Cytokines
- Chemokines
- Metabolic factors
- Other regulatory factors

Does it make sense to look for immunologically based biomarkers in serum (not liver) to correlate with HBV outcome and therapy?

Read about it

Hear about it

Check the Apps

Measure effects in blood
So, what can we measure in blood?

Circulating adaptive and innate immune cells:
• Phenotype and function
• Gene expression

Serum markers:
• Cytokines
• Chemokines:
• Immune regulatory factors
• Metabolic factors…
In CHB clinical course
- IFNα, IL-8 -> NK activation
- IP-10/CXCL10
- CXCL9/10/11
- IDO
- Arginase
- sPD-1

Outcome of antiviral therapy
- IP-10/CXCL10
- CXCL9/10/11/13, IL21
- sCD14
Cytokines induced in CHB flare promote NK-mediated liver damage via TRAIL/TRAIL-R pathway

Increased serum IFNα and IL-8 and NK expression of TRAIL during CHB flare

IFNα increases TRAIL expression in NK cells

Increased TRAIL R2 expression in HBV+ liver

IL-8 increases TRAIL R2 expression in HepG2
CXCL10 or IP-10 (IFNγ-inducible protein 10 kD):
Correlations with hepatocellular injury, T-cell activation, HBV DNA and HBsAg

- **Hepatocellular injury and inflammation**: Positive correlations with ALT, HAI and PD-1 expression on T-cells
- **Antiviral**: Negative correlations with HBV DNA /HBsAg

Hou, J. Viral Hep 2013

Wang, Antiviral Research 2014
CXCL9, CXCL10, CXCL11 and IDO (Indoleamine 2,3 dioxygenase)

CXCL9-11: chemokines from hepatocytes or liver sinusoidal endothelial cells
  • A role in recruiting DC, NK and T-cells

IDO: inducible in epith cells and DCs by IFNγ/TNFα with regulatory and antiviral activities

14 CHB vs 14 CHB flare vs 25 AHB vs 14 healthy controls:
  • Increased serum CXCL9-11 (but not IFNγ, IL-10 or TNFα) in CHB with flares vs CHB
  • CXCL9 levels in CHB correlate with ALT and IDO activity (KTR)
  • Increased IDO activity during ALT flare in CHB
  • Co-cultures with NK and pDC enhances IDO activity and suppresses HBV replication in-vitro

Yoshio et al, Hepatology, 2015

R=0.9
P<.0001

R=0.7
P=.01

*KTR: kynurenine : Tryptophan ratio (IDO activity)
Reduced serum L-arginine due to increased arginase activity in CHB coincide with increased serum ALT activity

Serum L-arginine is depleted in CHB with elevated ALT

ALT flares in CHB correlate with increased serum arginase activity

L-arginine replenishment restores CD8 T cell proliferation in vitro

Arginase activity is greater in HBV+ than HBV- liver

sPD-1: soluble programmed death 1 receptor

- 213 CHB vs 61 controls examined.
- Greater sPD-1 levels in CHB compared to NC with significant positive correlations with: ALT, AST, Tbili, HBV DNA, APRI, Fib4 (negative correl for platelets)
- Increased sPD-1 level with greater histological inflammation and fibrosis

Zhou et al, JVH 2019
Outcome of antiviral therapy

- IP-10/CXCL10
- CXCL9/10/11/13, IL21
- sCD14

In CHB clinical course

- IFNα, IL-8 -> NK activation
- IP-10/CXCL10
- CXCL9/10/11
- IDO
- Arginase
- sPD-1
Serum IP-10/CXCL10: Correlation with HBsAg decline during nucleoside/nucleotide therapy

- 126 CHB patients: 95 on NA therapy 6-107 months
- IP10 level at baseline and at virological response (<100 IU/ml):
  - Significantly greater in patients with >0.5 log HBsAg decline

Jaroszewicz/Cornberg, Antiviral Research 2011
Serum IP-10/CXCL10:
Decline in serum IP-10 on therapy correlates with virological response

Sonneveld, J. Hepatol 2013

HBV DNA decline
HBeAg decline
HBsAg decline

IP10 <150 pg/ml
IP10 >150 pg/ml

PEG IFN

Wang, Antiviral Research 2014

IP-10 pg/ml

On PEG IFNα  Off PEG IFNα

HBeAg clearance  HBeAg persistence

HBsAg decline > 1 log  HBsAg decline < 1 log

Telbivudine x 52 wk

Hou, J. Viral Hep 2013

Sonneveld, J. Hepatol 2013
Serum levels of CXCL9-11, CXCL13 and IL-21 correlate with HBsAg loss in AHB and in CHB with therapy

- Elevation of CXCL9-11, CXCL13 and IL-21 in acute hepatitis B (AHB) with subsequent HBsAg decline.
  - 41 self-limited vs 8 chronic evolution in AHB
  - IL-21 elevation observed only in resolving AHB patients, not non-resolvers.
- 2 CHB patients on NUC->PEG IFNα therapy:
  - CXCL13 and IL-21 elevation in 1 patient with HBsAg seroconversion, but not the other
sCD14: A marker of monocyte activation—increased with PEG IFN Tx and associated with HBeAg loss (? Also associated with cirrhosis progression?)

Increased sCD14 on PEG IFN therapy

Fold change in sCD14 +/- HBeAg loss at 26w post-Tx

Fold change in sCD14 +/- HBeAg loss at 26w post-Tx

Dou et al, JVH 2019

Sandler et al, Gastro 2012
HBV persists with global and virus-specific adaptive immune dysregulation

**Adaptive Immune Dysregulation**
- IFNα/ TNFα
- CD8
- CD4
- **↑** immune regulation:
  - PD-1/PDL1, CTLA-4, Bim...
  - Regulatory T-cells
  - Regulatory cyto/chemokines
  - MDSC -> Arginase
  - Metabolic deficit
  - HBV Ag

**Innate Immune Activation and Inflammation**

- pDC
- IFNα
- NK
- ↑ HBV
- KCs
- IL-8
- ↑ hepatic TRAIL R2
- Hepatocellular injury via NK TRAIL/ TRAIL R2
- NK lysis
- HBV-specific T cells
- Platelets
- LSEC
- HSC
- gMDSCs
- (Arginase, ROS, IL10...)
- Tregs
- IFNα, IL-8
- IP-10
- CXCL9-11
- IL1β, IL-10, IL-6, IL-18, TNFα...
- Arginase
- sPD-1
- sCD14
- CD8, CD4

**Inflammatory NK**
- TRAIL/NKG2D, cytotoxic
- CXCL9-11, CD38

Summary and Future Direction

• Numerous immunologically based serum biomarkers have been examined with correlations to clinical status and treatment outcomes.

• Despite their rationale, it is not clear that any specific immune-based biomarker is superior to available clinical and virological measures.

• Further studies are needed, including those evaluating the intrahepatic immune environment.
Thank you for your attention!